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THE EARLY DEVELOPMENT OF GAR-PIKE AND STURGEON.

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A MORE PERFECT KNOWLEDGE of the development of the Ganoids seems to be especially needed to enable the relations of the Teleostomes to be better understood. The study of fossils has at the present time given the materials for the better understanding of many important questions[1] relating to the descent of fishes, but its results must yet conform with those of the embryologist. It is thus especially unfortunate that the development of Polypterus is at present totally unknown, and that the study of even the more accessible forms, Amia and Polyodon, has as yet been neglected. Indeed, of those Ganoids which have hitherto been studied, the developmental history seems to have presented so many difficulties to observers that many years must go by before it may be satis-

[1] As for example, the relations of early Teleostomes to a stem essentially Elasmobranchian ; the descent of Mesozoic types of Ganoids from Crossopterygians; the lines of ganoidean specialization; the evolution of Sturgeon from palaeoniscoid; the affinities of physostome and caturid.

factorily understood. Every investigator who has been fortunate enough to secure the spawning fish, seems to have either failed in completing his material of developmental stages, or by faulty methods of preserving it has been unable to carry out a detailed study. The Sturgeon has not unnaturally been made an object of national investigation by Russians, and our present knowledge of its early development is to be obtained only in the more detailed works, in Russian, of Salensky ('77–'81) and Peltsam ('87). In Germany the studies of v. Kupffer ('91) on Acipenser were based upon material which unfortunately was lacking in younger stages. Of Lepidosteus embryonic stages, as is well known, have been obtained by different investigators five or six times, and within comparatively recent years: it has been owing mainly to technical difficulties that little has yet been published of its earlier developmental history.

In the following paper it is the writer's object to describe the early development of Gar-pike and Sturgeon, and by examination of these forms side by side to permit more definite comparisons as to the mode of development of Ganoids.

The material for the study of Acipenser was obtained by the writer during the spring of 1893 at Delaware City, Del., and has afforded a satisfactory and well-preserved series of general developmental stages. The mode of acquiring it has been already recorded[1]; spawning fish were taken several times during the writer's visit, and a number of experiments were made to determine a successful mode of artificially fertilizing the eggs, and a practical method of hatching them. As a result of the experiments it became possible to obtain an abundant supply of embryos, and considerable care was taken both to secure as perfect a set of the developmental stages as possible, and to preserve them in several reliable ways. As a fixing agent, the picro-sulphuric mixture should be mentioned as having proven in the main most satisfactory for sectioning. The developmental stages of Lepidosteus, as will be noted, were recently obtained during a visit to Black Lake, St. Lawrence Co., N. Y., made in company with Prof. E. B. Wilson, of

[1] Dean: Sturgeon Hatching on the Delaware. *U. S. F. C. Bulletin*, 1893, No. xviii.

Columbia College, who had undertaken to study the Gar-pike in the lines of experimental embryology. The themes of the present paper are arranged as follows:

I. The breeding and feeding habits of Lepidosteus.

II. The early development of Lepidosteus, — more accurately, that beginning with segmentation and ending with establishment of organs.

III. The early development of Acipenser, parallel in discussion with that of Lepidosteus.

IV. A general comparison of the early stages of these kindred forms, and a discussion of the developmental relations of Ganoid, Elasmobranch, and Teleost.

I. Mode of Occurrence of the Gar-pike; its Feeding Habits and Spawning.

The spawning habits of the Gar-pike, *Lepidosteus osseus*, have already been recorded by Garman ('78), Beard ('89), and Mark ('90). Their observations had in all cases been made at Black Lake, St. Lawrence Co., N. Y., the locality which has now acquired a well-merited reputation as a spawning region of this remarkable fish. To their accounts the writer would add the following notes collected at the same locality during the present season as preliminary to his discussion of the fish's early development.

The Gar-pikes of Black Lake are exceedingly abundant, and on account of their peculiar breeding habits there is little difficulty in securing their developmental stages. For several weeks during the spawning season they appear in shallow water, often in great numbers, and may be observed as they deposit their eggs. Matured fish, to yield eggs and milt for artificial fertilization, are then to be readily taken.

The general disappearance of the fish shortly after spawning is doubtless the reason that so little is known of its usual life habits. Garman states that after the spawning season the fish are "seldom seen, remain in the deeper parts of the lake away from shore, and are more or less nocturnal in habits.

Occasionally one may be taken with a minnow bait." This note, as far as the writer is aware, is the only definite reference that occurs in literature as to the usual habits of the fish. Belief, however, is very current that Gars are exceedingly voracious, killing and eating the larger fish, and not hesitating to attack even a swimmer who has ventured in their neighborhood. In the South, where examples have been taken as large as seven feet in length, their shark-like fierceness is credited more plausibly, and certainly to a degree which prevents the negroes from bathing in waters (*e.g.*, several branches of the Edisto River) where Gars are known to be large and abundant.[1] It is also currently believed that Gars are migratory, appearing at different places at different times, an idea probably to be traced to the general appearance of the fish only at spawning time.

Black Lake, however, furnishes conclusive evidence that the Gar is in no way migratory. The lake is but a small body of water whose communication with the St. Lawrence has long been cut off, and, though land-locked, has afforded especially favorable conditions for every life-stage of the fish. Their disappearance after spawning is, moreover, by no means complete. As stated by Mr. H. J. Perry, who for many years has carefully observed the fish at Black Lake, they may be seen near the surface at any time during the summer and fall. As a rule, their feeding habits appear then nocturnal and numbers are usually taken by night lines.

The actual mode of feeding has been observed by the present writer. The Gar approaches its prey (young dace) cautiously, advancing without perceptible movements; when within three or four feet it pauses, as if accurately to direct its aim, then, without seeming effort, it shoots quickly forward, secures the fish, stops suddenly, and is again motionless. An occasional bending of the head adds not a little to the apparent dexterity of movement. The food in all cases examined consisted exclusively of small soft finned fishes, mainly dace, none of which were

[1] The writer, while in South Carolina, was unable to obtain any direct evidence of the Gars' attacking even larger fish, nor has he seen any efforts on the part of specimens he has taken to warrant any belief in their fierceness.

longer than three and a half inches ; young perch and sunfish, abundant in the locality, do not appear to be eaten. The number of small fishes each Gar had taken was especially large and fully justifies the idea of the fish's rapaciousness ; from the stomach of a male (24 in.), caught while spawning, eleven cyprinoids were taken; of another (27 in.) the stomach contained the remains of thirteen fishes, while in addition three (of 2 in., 2½ in.) were taken from the pharynx. From all observations it would appear that the Gar is reasonably to be looked upon as only indirectly injurious to food fishes — *i.e.*, bass, perch, pickerel, pike, catfish — in reducing the general food supply. It is also worthy of note that there appeared throughout no evidence of the fish's having taken food that had been torn or cut ; the function of the straight, close set teeth seemed rather to prevent the escape of the prey than to kill or cut it.

Gars are extremely tenacious of life. Spawning fish taken by snare remained out of water two hours in the bottom of a boat : they were still alive and active, and supplied eggs and milt for artificial fertilization. Others after a similar journey were tethered by wire through mouth and gills and were kept alive for a week or more during the remainder of the writer's stay. Fish that had been speared and later placed in water remained alive for four or five hours. The strength of the fish is remarkable. This is perhaps in no way more evident than in the movements of swimming — its power to advance rapidly without apparent effort, to change at will from a position of rest to one of most rapid motion, and then to be able to regain instantly its position of rest. When taken from the water a large fish (4 ft.) in its efforts to escape can with difficulty be held in the hands. Its movements are varied and exceedingly strong, bending both horizontally and (slightly) vertically, almost serpent-like in its flexures. The movements of the head are especially noteworthy.

At Black Lake, as elsewhere, it is only at the time of spawning that Gars become noticeable. They are seen by the fishermen during the early part of May rising about their boats, thrusting their jaws out of water, often making a marked

"smacking" sound as they emit bubbles of air. Their appearance is at first general, notably in the regions of the deeper parts of the lake. Soon afterward they are seen basking near the surface, moving slowly away as a boat comes within a rod's distance. They are next noticed in schools of often twenty or more, sunning themselves in the middle region of the bays in whose shallows they will later spawn.

Spawning Habits.

The season of spawning appears a little more extended one than is usually stated, beginning about the middle of May and ending about the fifteenth of June. During this time spawning takes place intermittently, so that in all there may not be more than six or seven days in the entire actual 'run.' According to Mr. H. J. Perry there is usually an earlier and a later 'run'; the former occurs at favorable localities, induced by unusually warm weather, and lasts but two or three days; while the general spawning occurs about three weeks later. The variation in the time of spawning as far as the writer knows is indicated in the following table:

		Gars First Noted.	Spawn (and disappear).	Reappear.
1878	Garman	May 14	May 18–19	May 31–June 2
1883	Mark	?	June 10–12 (?)	
1884	"	?	June 3–5 (?)	
1888	Beard	?	May 24	June 8, 9
1893	H. Virchow	?	June 8–10 (?)	
1894	E. B. Wilson and Dean	May 3	May 14–18 and 24	June 10–12

Water temperature has doubtless with Gars, as with other fishes, an important relation to the time of spawning. The temperature of the shallow waters where spawning was taking place during the present season varied from 66° to 70° F. It is to be noted that the most active spawning occurred when the temperature was as low as 66° (Lower Deep Bay). Coolness in air temperature together with strong winds was found to have no immediate effect on the spawning fish, and even during a heavy cold rain-storm spawning was found not to

be discontinued. The writer notes that spawning occurs not merely "during the heat of the day between 12 and 3 o'clock," [1] but was observed at intervals from 8.30 morning to 7.30 night.

Especial localities have long been noted by the fishermen of Black Lake as favorable spawning grounds of the Gar, and certain particular shore spots or "points," often of but a few feet in diameter, as those in Upper and Lower Deep Bays, have been found year after year, to receive the first eggs deposited during the season. Later, when the height of the breeding season arrives, the fish may be seen spawning on almost every shore of the lake. "Points" at which early spawning occurs are hardly such as the term usually implies. They are little more than rocky shore strips, or rather particular portions, two or three yards in diameter, of a rocky shore. They are by no means prominent, nor are their rough rock fragments cleaner apparently than those of a part of the shore but a few yards away. There are thus but three spots in Lower Deep Bay where the eggs of the Gar are deposited, although the natural characters of the rocky shore are apparently uniform from the mouth to the head of the bay.

The behavior of the fish when spawning is worthy of especial note. When approaching the shore they are seen to be already divided into parties, each female readily recognized by her larger size (about 3 ft. 6 in.; 4 ft. 6 in.), attended by several males (from two to eight). All are pressed closely together, and in the slow advance and circlings of the party scarcely a movement can be detected. The snouts of the males, lighter in color, probably in sexual coloration, may be seen pressed under rather than over the sides of the female. All fins are spread, dorsals and anals widely erect, the former, together with the upper half of the tail, often protruding from the water and recognizable several rods from shore. The fish in the meanwhile enter very shallow water (five or six inches), so shallow in fact that the backs of the spawning fish are sometimes exposed. The arrival of the fish is followed by a period of quiescence; then after slowly moving to and fro, circling nearer and further

[1] Beard, ref. 6.

from shore, a few minutes later a sudden and active splashing takes place. A number of eggs have at that moment been scattered and fertilized, and the water is for the time clouded with milt. There will then follow a period of quiescence, often many minutes in length, followed by circlings and a second oviposition. The eggs, it may be noted, are not deposited at a particular spot, but appear to be sown evenly over the general spawning ground. It is also to be noted that during subsequent circlings of the female several males may often await her return, and from their movements it is not impossible that they are still emitting milt over the freshly deposited eggs. In no case was observed evidence of rivalry among the males, and an examination of all taken during the writer's visit could not detect any injuries caused by fighting. Their breeding colors are, however, prominent, and the richly pigment-spotted sides are accented by the bold markings of anal, dorsal, and caudal, made very evident by the habit of erecting the fins. The paired fins, too, appear of selective importance, and are widely spread during mating. Each is centered with a large ash-colored spot, especially prominent when seen under water. And the writer has noted that a male when unable to secure a place near the female has swum backward before the party, expanding his fins to the utmost, and making side and upward motions with head and paired fins.

It is probable, from observation of the spawning fish, that all eggs are not deposited by the female during a single day. Spawners were noted which deposited four or five batches of eggs and which then did not reappear during the day. Examination of the ovaries shows that but small portions of the eggs have at one time become detached from their follicles, but that the ripening process appears in every, and not a particular, region of the ovary. With Gar, as with Sturgeon, it seems evident that a large proportion of the ovarian eggs may not be duly ripened. Many examples were found whose abdominal cavity contained in quantity flaccid and slate-colored eggs which proved incapable of fertilization.

The fishes when actually mating are not easily alarmed. They may be approached closely, and even touched with the

hand. At the height of the spawning season large numbers (thirty) have been snared and speared at a single locality with but temporary alarm to the rest. Early in the season, when the fish were scarcely ripe, the writer found, however, that rarely more than two or three could be taken before the disappearance of all, — at least for a period of several hours. And the snaring of a female was found to give a greater alarm than the removal of several males. The note of Garman as to the cautiousness of the fish in scouting to determine the whereabouts of an enemy was fully confirmed. Timidity of the fish seems, moreover, indicated in the writer's attempt to lure male fish by means of a recently captured spawning female tethered over the spawning bed. Males were shortly attracted, but after one had ventured for a moment close to the female a general alarm was taken, and the fishes did not return.

The eggs almost immediately after fertilization become, as Garman and others have noted, excessively sticky, and acquire firm attachment wherever they happen to lodge, on stones (mainly), sticks, or water weeds. Over these collectors they are found well scattered, as the accompanying drawing (Fig. 1)

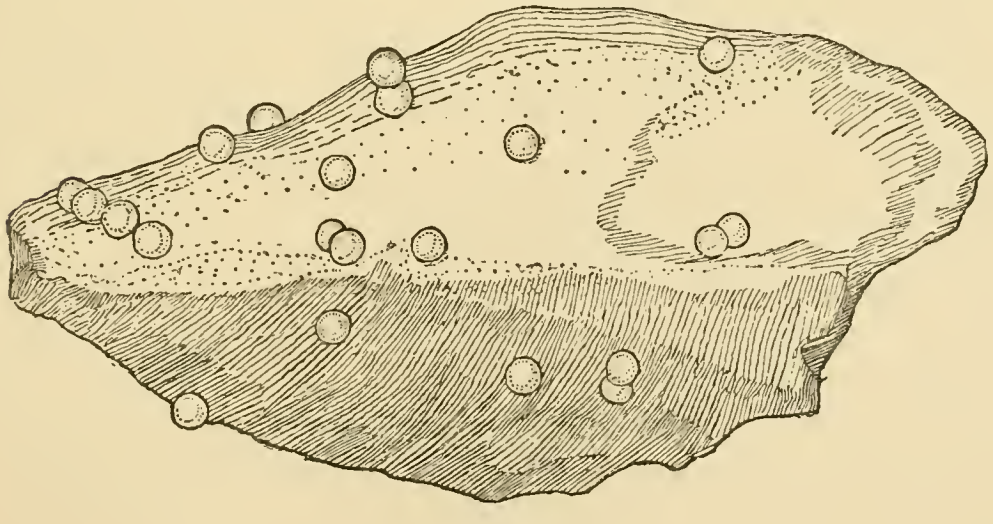

Fig. 1.

indicates. The stone fragments, loosely piled together, serve to attach eggs on every face, and not infrequently their under sides, being the cleanest, collect the greatest number. The

eggs are in fact sifted among the crevices of the rocks, and are to be found many tiers below the uppermost layer. The slime-covered character of the rocks seems little favorable to the prolonged fixation of the eggs, and within two or three days the majority become detached and are sifted deep among the rocks.

The color of the eggs at the time of extrusion is like that of the sturgeon's, slaty gray, and is strong in color contrast to the green-black rocks. About three hours later (early segmentation) the eggs become creamy yellow; two days later (end of gastrulation) the color has changed to a dull shade of greenish brown, although part of its duskiness may be due to the sediment collected on the outer membrane. The lightness in gravity of the animal-pole is early apparent, and when the axis of the eggs is disturbed the germ-disk regains its uppermost position in a surprisingly brief time, very much as in Teleost or amphibian.

As to the means adopted for securing embryological material; former experiments (Garman, Mark) had shown that egg-bearing rock fragments taken on the spawning ground might be retained in pans of water (renewed twice daily), and the eggs successfully hatched. A more convenient method was that of detaching the eggs from the stones while at the spawning ground and hatching them subsequently in earthenware dishes (Virchow[1]) or in a salmon hatcher (Beard). These plans were found by the writer safe and convenient, none the less because the natural hardiness of the eggs rendered them little subject to attacks of fungus (*Achlya*): and by their use there could certainly be no simpler way of securing developmental stages, were it not that a single objection proves most important: the eggs when collected are found to be of different ages, and in this confusion cannot well be reduced to a complete series of stages. By use of artificial fertilization, on the other hand, the writer found that every stage could be secured conveniently and in suitable quantity.

Artificial fertilization was first attempted at Lower Deep Bay, the writer employing the same mode of procedure and

[1] According to Mr. Perry.

the same hatching cases (v. *U. S. F. C. Bull.*, 1893, No. xviii, pp. 336–337) as he had used at Delaware City in the hatching of Sturgeon. Fish were freshly captured: the eggs were extruded by pressure and received in an earthenware dish. Here they were retained dry and shortly fertilized by a few drops of milt from a ripe male. They were then stirred; several minutes later water was added and as the eggs became adhesive they were quickly spread under water over netting-covered[1] hatching-trays. The thick, glairy mass which surrounds the eggs of Acipenser was not noted. Attachment was speedy; and the egg-covered trays, taken out of water, were taken back to Edwardsville — a two hours' row — with no further precaution than that of moistening the trays to guard against the drying of the eggs. This mode of transport the writer notes to emphasize the hardiness of the eggs, since it was later found that the proportion of eggs lost on these trays was scarcely greater than of those which were at once placed and retained in the floating hatching-boxes.

Artificial fertilization was repeatedly tried and always with favorable results; for this purpose fishes were transported living from the spawning grounds to Edwardsville. In obtaining the eggs it was found most convenient to excise the entire ovaries; from these the eggs were readily taken, and though not appearing to separate freely from their follicles they proved nevertheless capable of fertilization. The hatching boxes with their enclosed trays were floated near the shore in about five feet of water at the end of the wharf of Mr. Perry. Here the water was muddy and far from pure, but the eggs proved hardy and the loss from fish fungus was usually inconsiderable. On a tray where a loss of about 50% of the eggs was recorded, the mortality seemed mainly due to the crowded condition of the eggs. For convenience in securing developmental stages a mass of eggs were scraped from the trays and kept in the laboratory in earthen pans until required. As a fixing reagent (alcoholic and aqueous) picro-sulphuric acid was

[1] In his present experiments the writer employed "canopy netting" for the hatching frames. This material is of much finer texture than "mosquito netting," and its circular perforations seem more favorable for the attachment of eggs.

employed: this had been found to give the best results of all reagents the writer had used with eggs of Acipenser.

During development the water temperature varied from 62°–72° F. Hatching occurred in 200–220 hours.

In the following table the writer records the time occupied in the growth stages of Lepidosteus compared with that of Acipenser: the more rapid development of the latter was doubtless due to a slightly higher mean temperature of the water as well, perhaps, as to a smaller amount of food-yolk. At Delaware City the mean temperature was about 68°, while that of Black Lake was about 64° F. The writer has learned recently from Mr. Perry that eggs of Lepidosteus deposited on June 10 hatched by favorable water conditions in as short a time as 125–130 hours.

AFTER FERTILIZATION.	(*Hours.*)	
	LEPIDOSTEUS.	ACIPENSER.
First cleavage	1	1
Second cleavage	2	1.25
Third cleavage	3	2.30
Blastoderm cap grows down to the level of $\frac{1}{3}$ diameter of egg	32	16.30
" " " $\frac{1}{2}$ "	38	24
" " " $\frac{2}{3}$ "	42	26
" " " $\frac{4}{5}$ "	44	27
Gastrulation begins	37	19
Blastopore closes	46	58
Embryo appears	60	31
Central nervous system outlined	70	43
Pronephic ducts appear	70	42
Brain vesicles distinguishable	80	46
Optic vesicles distinguishable	80	46
Embryo surrounds circumference of egg 90° (approximate)	70	33
" " " 110° "	85	43
" " " 140° "	110	49
" " " 180° "	125	53
" " " 200° "	140 +	56
" " " 240° "	146 +	61
" " " 280° "	152 +	68
" " " 320° "	160 +	76
Movements of embryo first noted	149	82
Embryo hatches	200 +	94
Yolk material absorbed	1000 +	250 +

The accompanying figure (Fig. 2) shows in side view the natural position assumed by the embryo of Lepidosteus during its development: *a*, *b*, and *c* represent early stages of segmentation; *d*, early growth of blastoderm cap; *e*, *f*, and *g*, gastrulation; *h*, early embryo (the heart region is now at the animal pole of the egg and the blastopore in a position a little below the equatorial line); *i*, embryo of 92 hours (sagittal axis of embryo has now become horizontal); *j*, the horizontal projection of the same embryo; *l*, embryo of 130 hours (region of embryo

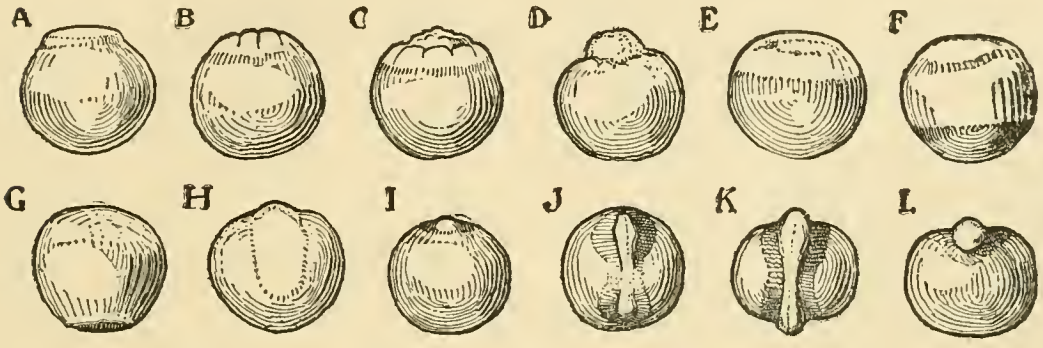

Fig. 2.

near pronephic tubules is now uppermost); *k*, same in horizontal projection. The positions assumed by the developing Acipenser are essentially the same as in the above figures; the only stage of which the author is doubtful is that of the early embryo, *h*.

The eggs of Lepidosteus and Acipenser compared with those of Teleosts are exceedingly large in size, and in their pigmented character and mode of early development they are strongly suggestive of amphibian. The following comparison may be given of their general characters: —

EGG.	SIZE.	ADHESIVENESS.	MODE OF DEPOSITION.	YOLK AND CLEAVAGE.	PIGMENTATION.	MICROPYLE.
Gar	3.3 mm.	Adhesive immediately after fertilization; becoming attached, often remain thus until hatched.	Sown thickly over rocky fragments, rarely found clustered in numbers.	Large in amount, evenly distributed, cleavage unequal, meroblastic in character.	Slightly pigmented when deposited; acquires grayish pigment during development.	Single.
Sturgeon	2.9 mm.	Slightly viscid after fertilization; give off in quantity a glue-like substance; become attached in about twenty minutes; do not become separate.	Scattered on shelly bottom; found attached in strings and flattened masses to shells and sticks; rarely separate.	Little yolk, cleavage unequal and holoblastic.	Richly pigmented, brown, in and encircling germ area; under pole evenly but less darkly pigmented.	Varies in number from 3 to 9.

II. The Early Development of Lepidosteus.

The egg after being deposited becomes pale cream yellow in color, and for a short time does not exhibit the protoplasmic germ area. The condition of its membranes has been made the subject of the exhaustive research of Mark, who figures and describes for the first time the single micropyle and its contained plug of granulosa cells. The maturation of the egg has not been sufficiently followed by the writer to warrant discussion; he notes that the egg at the moment of extrusion shows a well-marked maturation spindle in the immediate neighborhood of the micropyle, of which Mark has given an excellent figure; a polar body is here given off almost immediately after fertilization; its appearance is similar to that figured by Böhm[1] in trout; it has not, however, like the latter, been seen to undergo a second division.

The change in the egg-substance which permits the heavier yolk to sink to the lower pole of the egg is not apparent to the eye during the first ten minutes after fertilization; the lower hemisphere is then noted to be of a darker color. Soon afterward the egg presents somewhat the appearance of Pl. I, Fig. 1, — its germ area, however, is somewhat larger than figured and its marginal limit less clearly marked. The egg one hour after fertilization, seen in the figure, is on the point of undergoing the first cleavage: its germ area is roundly oblong, of flattened surface, slightly raised above the yolk. Sections, however, indicate that at this stage germ disk and yolk are more intimately connected, and in Pl. II, Fig. 21, it will be seen that the protoplasm of the animal pole is hardly to be separated from the coarse granular yolk until nearly in the equatorial region of the egg. In the figure the nucleus is seen dividing at about one third the diameter of the egg from the surface.

Segmentation.

First Cleavage (Pl. I, Fig. 2). — The first segmentation furrow appears in the living egg as a thinning away of the germ

[1] A. A. Böhm: Die Befruchtung des Forelleneies. *Sitz.-Ber. d. Gesell. f. Morph. u. Physiol. München.* 5 Mai, 1891.

disk in a vertical plane; it results in a trench-like groove showing the yolk mass below separating the blastomeres; at either end it bows outward, rounding the corners of the germ disc, and extends no further down the side of the eggs in any example the writer has examined; the margins of the furrow are boldly marked, highest at the animal pole of the egg. Sections at this stage indicate that the germ disk is more clearly to be distinguished from the underlying yolk, the nuclei occupying a relatively higher plane than in the preceding figure; the furrow is seen (Pl. II, Fig. 22), to leave below it undivided a well-marked layer of the germinal protoplasm. The first furrow has been observed[1] to divide the germ disk into segments of unequal size, an abnormality of cleavage well-known in Teleosts,[2] which was found to influence in no way subsequent development.

Second Cleavage (Pl. I, Fig. 3), in all cases examined, occurs in a vertical plane approximately at right angles to the first. It is expressed in the germ disk only, and like the former furrow could not be traced in the yolk region of the egg. The polar corners of the blastomeres are the most prominent, sharply cut, and but slightly rounded. The nuclei remain in the horizontal plane of those of Fig. 2, and no change is seen to occur in the layer of protoplasm underlying the furrows. During cell division the nuclear changes are not prominent: the spindles are seen with difficulty, the chromosomes are small and obscure, and in the resting stage the outline of the nucleus can hardly be determined. In general the position of the dividing nuclei with respect to the blastomeres is similar to that of amphibian: shortly after division the nuclei are seen to be separated by a thick transparent disk of protoplasm, a segmentation plane which is later expressed in the surface furrow (Pl. II, Fig. 23).

Third Cleavage (Pl. I, Fig. 4) is also in a vertical plane: in general its direction is parallel to the first furrow;[3] often,

[1] By Prof. E. B. Wilson.

[2] *E. g.* Ryder in the Cod and H. V. Wilson in Serranus.

[3] This was accurately determined by E. B. Wilson in his experiments upon eggs attached to glass plates whose various cleavages were recorded.

however, as seen in the figure, it diverges somewhat, tending to appear meridional. In depth and lateral extension its furrows are entirely similar to those of earlier cleavage. The position of the nuclei in this stage is shown in Pl. II, Fig. 24. It will be noted that the blastomeres are now more widely separated by the first and second furrows ; these interstices are the first indications of the segmentation cavity. A view of this stage as seen from the side is given in Pl. I, Fig. 5.

Fourth Cleavage (Pl. I, Fig. 6) is again a vertical one ; it is in general parallel to the second furrow, resembling in other regards the third cleavage. The extent of the cleavage fissures may be made out in Pl. II, Fig. 25, a section in which several nuclei are to be seen dividing for the fifth cleavage. During this stage many variations occur in the size and shape of the blastomeres; they may readily be reduced, however, to the normal plan of segmentation. A vertical section of this stage (Pl. II, Fig. 26) shows the depth of the furrows, and indicates as well the relation of blastodisc to yolk.

Fifth Cleavage (Pl. I, Fig. 7), results in a normal stage of thirty-two cells : it is carried out often with wide variations, and the lineage of the blastomeres is to be followed only with difficulty. By serial sections of the more regular examples of cleavage in the late 16-cell stage, the nuclear figures (as for example those of Pl. II, Fig. 25) permit, however, the ideal plan of the fifth cleavage to be understood. This the writer expresses in the accompanying figure (Fig. 3), in which is indicated the lineage of eight, sixteen, and thirty-two blastomeres. Those derived first from the four original blastomeres are denoted by *a*, those from their derivatives by *aa*, those from their derivatives by *aaa* ; a second derivative from the original blastomere is denoted by *b*, a third by *c*, and

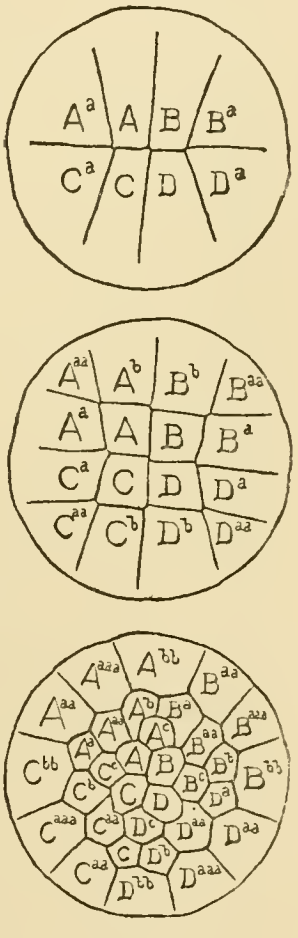

FIG. 3.

their subsequent derivatives, as *bb*, *bbb*, or *cc*, *ccc*. The writer believes, accordingly, that the plan of the fifth cleavage is carried out in the marginal cells' budding off their polar ends, giving rise to a circle of twelve cells ; the four cells of the animal pole dividing obliquely in a meridional plane. This mode of fifth cleavage corresponds clearly with that of the ideal type in Teleost segmentation (H. V. Wilson). In many examples of this stage the present writer notes that the four cells of the animal pole undergo horizontal cleavage, so that in surface view but twenty-eight blastomeres may be counted.

From the fifth cleavage onward the division of cells could not be satisfactorily followed, and the outward appearance of similar stages presents so many variations that they seem valueless to record. In the sixth cleavage (Pl. I, Fig. 8), the only cell divisions that were noted as generally constant were those of the marginal cells: these undergo meridional cleavage, similar to the former one, its furrows extending no further than the margin of the cell-cap.[1] It is in fact in this stage that the cell-cap is largest in outward size. Horizontal cleavage has taken place irregularly, and has caused the lower layer of the germ protoplasm to be broken up into irregular cells whose upper and marginal limits are readily traced, but whose cytoplasm is seen clearly in many cases confluent with the yolk. This layer of yolk cells is represented in the vertical section of the egg at this stage (Pl. II, Fig. 27). It will here be seen that the cell-cap consists of a loosely piled cell mass, of two (or three) tiers in depth, inclosing an irregular segmentation cavity. In the floor of this blastoderm cells are distinguished which below open into the yolk mass, and which, most important, may be seen splitting off nuclei into the yolk below. This process in the formation of merocytes the present writer regards as essentially the same as that occurring in Elasmobranchs, and believes that in it the ancestral

[1] The writer has found no segmentation stages in which the furrows extend much lower than the equatorial region of the egg. He does not, accordingly, confirm the note and figure of Balfour, and is inclined to believe that the total segmentation of Lepidosteus occurs only as a variation.

condition of the periblast (of Teleosts) is most nearly represented. In this regard he would call especial attention to the segmentation stages immediately succeeding this, for it seems to him evident that the marginal cells undergo a metamorphosis similar to that concerned in the formation of periblast which H. V. Wilson [1] has figured.

After the sixth segmentation continued divisions result in the production of a flattened cell cap (Pl. I, Fig. 9), irregular in surface and outline. Its marginal cells are irregular and inconspicuous, and their lateral angles obscure. The cell-cap increasing by continued horizontal division comes next to present an irregular but prominent summit ; its margin is irregular and slightly depressed. At a later stage (Pl. I, Fig. 11), the marginal depression has become converted into a moat-like groove. Cells are still seen prominently along its sides although at the summit of the cell-cap continued divisions have rendered them so small that their outlines can be discerned only by means of sections. The noteworthy point, however, at this stage is that at certain points of the outermost cell-ring the cell-walls are found to have entirely disappeared and the nuclei are seen to have entered into the peripheral yolk. This change will at once be seen to correspond closely to that in *Serranus* which Wilson has figured in his memoir, Figs. 21 and 22. At the same time, furthermore, a median vertical section of this stage (Pl. II, Fig. 28), indicates that it is not at the periphery of the cell-cap alone that this modification occurs ; the entire floor underlying the cell-cap is now smoothly and distinctly differentiated ; it is no longer filled with the rough and half detached cell outlines of an early stage ; the nuclei scattered through it are seen in continued division, occupying a deeper and deeper plane in the subjacent yolk, and budding off at the surface additions to the cell-cap only at irregular points. The cell-cap is now seen to have a thickness of four cells, and their loose clustering has given rise to an irregular segmentation cavity.

[1] H. V. Wilson, On the Embryology of the Sea Bass, *Serranus atrarius*. *U. S. F. C. Bull.*, No. IX, 1891.

Blastula.

A very late stage of segmentation is shown in surface view in Pl. I, Fig. 12, and in vertical section in Pl. II, Fig. 29. Contrasting it with the stage last figured, the following differences are apparent : the summit of the cell-cap is larger and rounder ; division has caused the remaining cells of its margin to become indistinguishable ; its outline has become sharply defined, and its margin, if anything, slightly projects above the marginal groove: of the latter the outer boundary cells have almost disappeared, and their nuclei are now found dividing and migrating into the underlying yolk. The cell-cap has now a thickness of seven or eight cells ; its segmentation cavity has become larger and more distinct ; its line of separation from the yolk is even clearer than in the former stage. At certain points, as before, it receives increments of cells from the underlying yolk. The yolk nuclei are now exceedingly numerous in both central and peripheral regions, and of them as many as four or five tiers may be counted. The entire periphery of the cell-cap is now clearly distinct and separate from the yolk.

Gastrula.

From these conditions the transition to early gastrulation is readily followed. In Pl. I, Fig. 13, the cell-cap of the former figure has flattened out as a disk whose margins bend around and enclose the animal pole of the egg. The boundary between blastoderm and yolk is clearly drawn, although at one side, the future tail region of the embryo, its demarcation is more sharply drawn. Pigment is now noticeable in the yolk region, and becomes still more evident as the blastoderm continues to enclose the yolk ; a light olive color is acquired which gives a sharper outline to the adjacent blastopore. A sagittal section of this stage permits the following growth changes to be noted : the cell-cap of Fig. 12 has resolved itself into a well defined roof for the segmentation cavity, forming a homogeneous layer of small cells, whose thickened margins are continuous

with the yolk ; at the animal pole it consists of about five tiers of cells, and of twice as many at its periphery (Pl. I, Fig. 30). At the posterior region of the blastoderm occurs the sharp surface indentation which marks the dorsal lip of the blastopore ; there is as yet, however, no fissure beneath its margin ; the floor of the segmentation cavity has undergone important changes ; it is now formed of a (single) layer of round, many-sized cells, which the writer has seen in cases budded off from the underlying yolk, but which he believes are mainly derived from the peripheral region, where cell division is now most active ; the line demarking the yolk from this layer is at nearly every point most sharply drawn ; below it, however, no prominent yolk nuclei are to be seen as in the former stage, but the entire upper region of the yolk seems to have acquired a quite different character ; it now consists of elements which suggest those of the white yolk of the chick.

A slightly later gastrula is figured in Pl. I, Fig. 14, a stage that is prominent on account of the indentation which the blastoderm shows in its hindermost margin ; it is here that the rim of the blastoderm is thickest and most sharply separate from the yolk ; its thickening is not widely different from the shield-shaped embryonic mass of Teleost, although wider and less evident. Sections show but slight changes from the earlier conditions ; in the margin of the blastoderm the cells have become more numerous ; the anterior lip is still closely in contact with the yolk, the posterior is free for a short distance under its immediate margin ; the embryonic thickening near its margin is due almost entirely to an increase in the number of cells of the blastoderm in what has now become the axial region of the embyro.

In Pl. I, Figs. 15 and 16, are figured two later gastrulas : the yolk is in these only to be seen through the rapidly constricting blastopore, which in its latest stages is usually circular.[1] In the first the blastopore is already circular, all traces of the marginal indentation of Fig. 14 having disappeared ; this was observed to take place not by the concrescence of the

[1] The writer was first led to suppose that the circular type of blastopore was abnormal ; this he now believes is the usual form at this stage.

sides of the indentation, but by a mode of unequal growth which caused the indentation to become less and less prominent until it disappeared. In Fig. 16 the marginal indentation has been retained and it is found to be lost only by the time the diameter of the blastopore is reduced about one-half.[1] In Fig. 16 the margin of the blastopore is seen to be prominent, and the outline of the embryo may be traced in the light-colored region anteriorly. A sagittal section of this stage, Pl. II, Fig. 31, shows the extent to which the lips of the blastopore are now separate from the underlying yolk; it shows clearly the point of union on either side of the yolk and the cells of the inner germ layer (* of the figure). Its coelenteron (*c*) is similar to that of amphibian, and the writer believes that its mode of growth will upon careful study prove corresponding. The section just noted shows in addition the boundary of the segmentation cavity (*s*), and around the blastopore the partial separation of the outermost epiblastic cell stratum, 'Deckschicht.' Of this the differentiation is later found extended over the entire surface of the egg. The yolk material slightly protrudes through the blastopore as a rounded yolk plug. No beginnings of the middle germ layer have as yet appeared.

From the conditions above described it seems to the writer that the gastrulation of Lepidosteus might be looked upon as an intermediate type, — primitive, moreover, inasmuch as from its beginning till the time of the closure of the blastopore but the two primary germ layers are concerned in its formation. It is intermediate inasmuch as it presents a striking similarity on the one hand to the gastrula of an Elasmobranch; on the other that it parallels the holoblastic type of amphibian, and that it further indicates in its structures the most essential characters of Teleost. It resembles the gastrula of Elasmobranch in (1) its meroblastic origin, (2) the presence of generally diffused yolk-nuclei in a granular superficial layer of yolk, (3) the sharply marked character of the yolk surface below the segmentation cavity, or more accurately beneath its

[1] Many variations occur; examples were noted in which the blastopore was elliptical, ovate, or even slightly constricted in its ventral margin.

irregular floor of loosely associated cells, (4) the even thickness, homogeneity, and sharp demarcation of its germ disk (outer germ layer), (5) the mode of its invagination, especially in the mode of union of the invaginated layer with yolk, (6) the formation of the indentation at the hinder margin of the blastoderm. In short, the addition of abundant yolk would seem in the opinion of the writer to render the type of gastrulation of Lepidosteus in every essential regard similar to that of Elasmobranch. On the other hand, the resemblances to amphibian gastrula might be looked upon as only superficial; its most decided similarity seems but the result of its approach to a holoblastic condition, as seen for example in the formation of a circular blastopore, and in the outward growth changes of the early embryo (90–130 hours). To the Teleost gastrula its resemblances may be summarized in (1) the origin of yolk nuclei from the marginal blastodisc (=periblast), (2) the plan of early segmentation, (3) the shield-shaped embryonic mass, and (4) as will later be seen, the absence of a true neurenteric canal.

The establishment of the outward shape of the embryo has its beginning in the stage figured from above and from the side in Pl. I, Figs. 17 and 18. The embryo is here a light-colored cell-mass, whose rounded sides slope gradually to the yolk below: a minute pit is the disappearing blastopore, marking the tail region of the embryo; the head end is sharply raised and slightly projecting; immediately in front of it a circular and somewhat depressed area denotes the *anlage* of the vascular system. A sagittal section of an embryo of a slightly earlier stage, Pl. II, Fig. 32, is worthy of careful study: it shows the condition of the closing blastopore, and the origin of the middle germ layer. According to the writer's observations the closure of the blastopore takes place in the following manner: at the stage figured in Pl. I, Fig. 15, the rim of the blastopore is becoming thickened and rounded, and the dorsal lip is already considerably the thicker; this mode of increase in the thickness of the blastopore rim is continued until its closure; at about this time the relative thickness of both poles may be well seen in the section, Pl. I, Fig. 32: the outer

epidermic layer (*ep*), 'Deckschicht,' now appears to aid in the process of closure; at the margin of the blastopore it thickens and appears especially distinct, and protrudes over the rim of the blastopore, as it does in fact in Teleost: its innermost cells, however, are still connected, but loosely, with those of the inner layer: the continued reduction of the blastopore now results in a funnel, closed at its pointed end by the inwedging epidermic cells; closure and fusion of the lips of the blastopore next follow by what appears a concrescence of the sides of the funnel, an obliteration completed as the caudal eminence of the embryo is in process of forming. It will accordingly be seen that the observation of Beard as to the absence of a neurenteric canal is thus confirmed.

The view of that author, however, as to the origin of the middle layer "from the epiblast on each side of the middle line, and from the epiblastic region at the lip of the blastopore" has not been verified. It will be seen in the above section that the mesoblast (*m*) is clearly to be traced to its union with the hypoblast (or more accurately, perhaps, the undifferentiated tissue) in the underlying region of the blastopore. Its growth thence extends on all sides, but most rapidly forward in the direction of the embryo's axis: in the transverse section, Pl. II, Fig. 33, gastral mesoderm is present; its connection with the inner layer was observed only in the hinder portion of the embryo.

In a sagittal section of an embryo of about 60 hours, Pl. I, Fig. 18, the region of the origin of the vascular system is of considerable interest. The outer layer, after its thickening at the head end of the embryo is seen suddenly to taper away as it slants downward continuously over the depressed area; it now consists of the epidermic stratum, which has already been noted, and an irregular single-celled layer of formative epiblast, and in this condition it is continued around the yolk, Pl. II, Fig. 34 (in this figure the drawing has not been accurately reproduced). The segmentation cavity was last seen in Pl. II, Fig. 31; its flattened condition in that stage has now given rise to the vascular disc-shaped enlargement immediately in front of the embryo's cephalic eminence; in other regions it has become

so exceedingly flattened that it can be discerned only with difficulty. The floor of the vascular enlargement is the region of especial interest ; it is already covered with spherical mesenchyme elements which are seen in process of being budded off from the yolk ; they have certainly this origin, although the writer believes that they may also be derived from the marginal cells of both the upper and lower layers : the conditions however that are here presented appear strikingly similar to those figured by Rückert in Selachian. The fate of these vascular elements will later be referred to.

Early Embryo.

The stage in which the outward shape of the embryo begins to appear is figured in Pl. I, Fig. 19. Its axis is almost a straight one, and the slender embryo is sharply constricted off with head and tail eminences especially prominent above the surface of the rounded yolk. The head eminence is the embryo's widest part, its outline is bluntly lanceolate, its dorsal surface is flat, but shows the presence of a faintly marked cord of cells in its median line. The tail eminence is less prominent ; it is well rounded, highest in its posterior part. The middle region of the embryo remains as yet at the egg surface. A slight rounding in of the surface at the sides of the embryo is the first indication of a parietal zone. The following structures take their definite origin during this stage : notochord, primitive segments, central nervous system and blood vessels. Of the pronephric duct early traces are found but its more definite appearance is at a later stage. The notochord takes its origin from the hypoblast in a manner very similar to that of the Teleost ; by the time the entoderm has spread under the embryo its thickening is noticeable in the axial line ; the ridge that is thus formed is more prominent near the hinder body region and it is here that the chord is first seen separate from the entoderm. Its appearance at this stage is shown in Pl. II, Fig. 33, *c*. In the embryo described four primitive segments are present ; the mode of their formation is to a degree shark-like inasmuch as the visceral and parietal

layers of the middle layer which form them are early separate, and as traces of a lumen in early segments are to be found. The solid character of the later muscle plates appears strikingly Teleost-like. These the writer believes furnished the basis of Beard's observations; they certainly appear, however, long before traces of the auditory capsules appear, and rather in the mid-region of the embryo's axis; their increase is then as Beard states at the expense of the hinder tissue.

The definite origin of the central nervous system does not appear in a stage much earlier than that of Pl. I, Fig. 19, and it is here only in the anterior region of the embryo that its relations may be determined. In the hinder trunk region the formative epiblast is found to be generally thickened but otherwise undifferentiated in the median line; in the mid-region its layer becomes thinner and flatter; in the head eminence, on the contrary, it becomes thicker, its boundaries are well marked, and it is deeply implanted in the median line; a median cell cord is now to be recognized as its outward expression; as yet a lumen has not appeared and the general appearance of the spinal cord is teleostean: no sense organs have as yet made their appearance. The prominent margins of the head eminence are formed of the anterior extension of the mesoblast; and the writer believes that it was this mesoblastic rim of the head eminence that Balfour and Parker have figured as the early brain vesicles. The writer notes that the anterior end of the central nervous system in this stage ends not in a point of contact with the cells of the ectoderm (lobus olfactorius impar[1]) but in a thin continuous cell sheet which underlies the ectoderm and forms the roof of the vascular area (segmentation cavity). The relation of the mesenchyme elements, which were earlier seen occupying the vascular area, to the formation of blood and vessels is by no means easy to determine; and the wide divergence among observers in their studies on the origin of the vascular system in well studied types seems to render inadvisable what can be but an imperfect note on the conditions of Lepidosteus. The writer would record, however, that as far

[1] As Kupffer has demonstrated in the Sturgeon, and as others have recently described in other vertebrates.

as his observations have extended, the characters of this form show a close agreement with those which Rückert has described in Selachians: the present writer finds, for example, that the vascular mesenchyme cells become early associated in loose clusters in front of the head region of the embryo, and these he has traced assuming definite shape as a blood tube; he notes that the early blood cells resemble closely the cells of the early mesenchyme and that these seem to have their origin in the anteriorly extending yolk-margin of the vascular cavity.

The early embryo has clearly attained its outward form in the stage figured in Pl. I, Fig. 20, in which seven primitive segments are present. The outline of its trunk has become constricted off from the yolk; the eminences both of head and tail are rounded and prominent, the mid-region of the trunk is well raised above the yolk, and its parietal lamellae are now sharply marked as a groove on either side of the embryo. In the dark lateral margin of each parietal layer the pronephric duct has become established. The advancing structures of this stage which are to be briefly noted are (1) the brain vesicles, (2) optic capsules, (3) the establishment of a lumen in the anterior nerve axis and (4) pronephric duct.

The changes of the anterior part of the central nervous system of a stage slightly later than this have been described by Balfour and Parker. The first appearance of a lumen in the embryonic brain is to be found in the present stage. The faintly marked axis of the head region denotes the wedge-like insinking of the brain region, and its irregular margin is at present the only outward indication of the vesicular enlargements. Sections show, however, that the *anlage* of the optic vesicles exists in a grouping of cells on either side of the axis at the broadest part of the cephalic plate; and further that the lumen which is found within the central axis first occurs in the mid-region of the brain, arising, as Balfour and Parker believed, from the disassociation of cells: that this cavity, however, occurs before that of the optic vesicles is demonstrable, and even in a stage 24 hours older than this. The English observers have figured a similar condition (*Ref.* 5, Pl. XXII, Fig. 26), although they state the lumen as there probably occurring,

but not to be seen, "the section merely passing through them to one side." The origin of the auditory invaginations has been well figured by Balfour and Parker: it is to be noted that their appearance is about 10 hours later than of the optic vesicles. The pronephric duct in its earliest stages seems to the writer more closely comparable with that of Elasmobranch than earlier writers have inferred: the cell end, budded out of the parietal middle layer was noted (in embryos of 11 somites) as at first solid[1] later acquiring its lumen through the disassociation of cells; its irregular ridge-line projecting towards the outer layer suggests closely a condition of *Pristiurus*, and its early connection with the epidermis in the region of the fifth somite is an additional character of comparative interest. The pronephric duct occurs early in development, before even the appearance of the lumen in the optic vesicle, or in the hinder neuron, and before the constriction off of any part of the gut, in strong contrast to its retarded development in Teleosts. The history of the pronephros, as given by Balfour and Parker, has not been followed in detail: this together with the discussion of the inner germ layer might, perhaps, be better followed in a study of the later development of the embryo.

III. The Early Development of Acipenser.

The egg of the Sturgeon when extruded is slaty-gray in color: its general surface is pigmented and a rich mass of pigment darkens the animal pole, often displayed as a cross, or star-like marking (Pl. III, Fig. 35). The germ-disc is already clearly outlined, and its lighter color is made especially prominent by an encircling darkly pigmented zone of the yolk.

The egg membranes at this stage may be compared with those of Lepidosteus as figured by Mark.[2] The zona radiata is similar in relative thickness, and the villous layer is clearly distinguishable, although staining but lightly. The outermost layer is distinctly separate from the others: as Salensky notes, it stains readily (but irregularly) with haematoxylin; it is many-layered and thick, often four times the thickness of the

[1] The present writer confirms the observations of Beard. [2] *Ref.* 17.

radiate and villous layers; its outer surface is irregular and raised in projecting bosses of many sizes. The outer layer, which may prove the equivalent of the *granulosa*, is clearly the adhesive envelope of the egg: before immersed in water its thickness is less than that of the combined inner layers; its structure is lamellar, and its specialized hygroscopic character is doubtless the cause of its cellular elements being difficult to distinguish. The glairy mass which the fertilized eggs give off after some minutes' immersion in water seems clearly the product of the outermost envelope. The writer, it will be seen, differs in the interpretation of the egg membranes from Mark, who regarded it "probable that the outer layer would be found to correspond to the villous layer of the Gar-pike," and "that the middle layer was simply the differentiated outer half of the zona." The present writer is, however, by no means convinced that the outer layer is to be regarded as the granulosa, although he regards it probable. During the later developmental stages, when partly detached and greatly reduced in thickness, it becomes most similar to the outer membrane of the Gar-pike: its irregular thickened and distended character during the period of the eggs' fixation may accordingly prove a specialization of its outermost layers to acquire an hygroscopic function, or may be even, but less probably, due to a direct secretion of its glandular cells.

The outward changes which the egg has undergone by the time of the first cleavage are not noteworthy. Sectioned at this state the egg presents but slight differences from the conditions of Lepidosteus, *cf.* Pl. I, Fig. 21, and Pl. III, Fig. 55. The line of demarcation of the deutoplasm is less clearly drawn and the deutoplasm itself is more coarsely granular, its elements usually containing a store of more finely differentiated yolk. The nuclei are seen dividing at a similar *niveau*, and are larger, though less distinctly marked. In preparations of all early stages the chromosomes are not readily determined. Certainly the most characteristic feature of the egg is the presence of abundant pigment: it is seen massed at the animal pole as if in a vortex, its granules sinking in thread-like clusters downward and outward.

Segmentation.

The *first cleavage* is vertical and occurs at about the same interval after fertilization as in *A. ruthenus*. As Salensky has determined, it separates the blastomeres only in the region of the germ-disc. It is deepest at the animal pole but traverses the blastodisc sharply, Pl. III, Fig. 36, causing a narrow surface fissure different from the trench-like furrow that has been noted in Lepidosteus. Until the appearance of the second cleavage its marginal limit is in the pigmented zone of the yolk bordering the blastodisc. The present writer notes that in transverse vertical section it differs little from that figured in Pl. II, Fig. 22; the furrow is more sharply cut and deeper but does not penetrate into the yolk. The pigment at the animal pole is now restricted to a circular area comparatively regular in outline. In the earlier stage the outline and extent of the surface pigmentation has apparently no influence in foretelling the direction of the first cleavage plane.

The *second cleavage*, again vertical, corresponds closely with that of the Gar-pike. It is approximately at right angles to the first plane, exceptionally oblique, as in the figure, Pl. III, Fig. 37, and may intersect the first furrow with noteworthy Polflucht. Its first outward appearance is in the region of the pole, thence it may be followed as it extends marginally. When it has traversed the blastodisc its depth is approximately that of the first furrow, although outwardly the latter is readily distinguished by its greater extent; it now surrounds (almost) the entire circumference of the egg, but in the yolk region, as Salensky notes, its furrow is of the most superficial character. The position of the nuclei in this stage is entirely similar to that of Lepidosteus, and has been figured in vertical section by Salensky, *Ref.* 29, Pl. XV, Fig. 7: his drawing illustrates the depth of the cleavage and the line of demarcation between germ and yolk. A horizontal section (slightly oblique) through the region of the nuclei in this stage is given in Pl. IV, Fig. 56; in this figure the distribution of the pigment is to be traced; at the surface it has accumulated in the rounded and

irregular corners of the blastomeres, and below the animal pole it has extended deep into the germ-disc.

The *third furrow*, Pl. III, Figs. 38 and 39, is of interest on account of its irregular appearance: although usually vertical,[1] parallel to the first plane of cleavage, it may be meridional and even strictly horizontal, with a range of intermediate variations. As a large proportion (about fifty per cent) of the eggs examined conformed to the plan of cleavage of Lepidosteus the writer believes that this is the normal mode of cleavage of *sturio*. It is first expressed, like the second cleavage, in the immediate region of the animal pole, thence extends both centrad and peripherad, but does not pass the limits of the blastodisc.

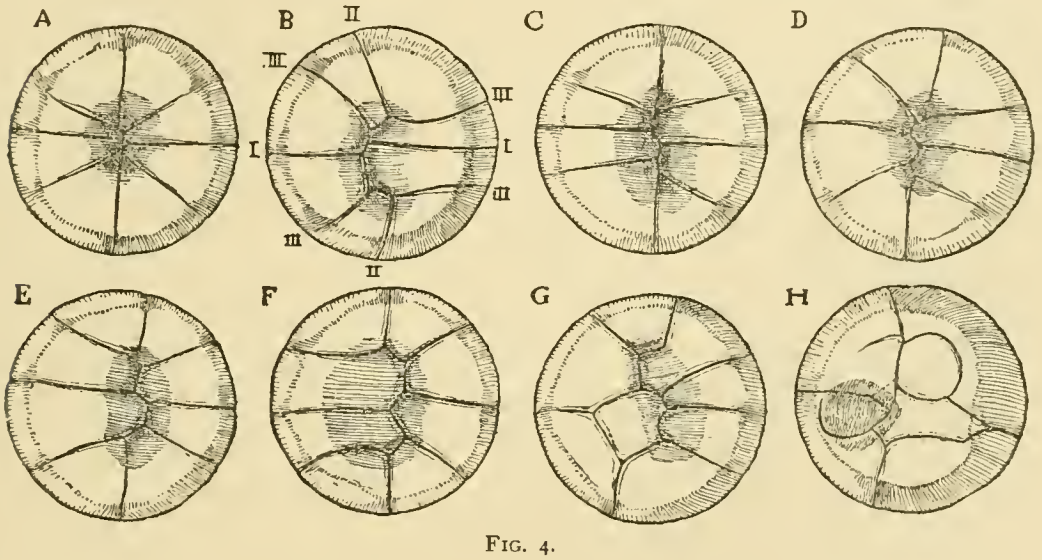

FIG. 4.

Marginally, however, it may attain the bordering pigmented zone. It is in general readily distinguishable from earlier furrows: the first cleavage has by this time completed its circle at the yolk-pole of the egg while the second has passed downward through the pigmented zone and is half surrounding the yolk mass. The *nivcau* of the nuclei and the general pigmentation do not differ from the earlier conditions. A horizontal section, Pl. IV, Fig. 57, may be compared with that of the eight-celled stage of Lepidosteus, Pl. II, Fig. 24. It will be noted that in Acipenser the pigment has penetrated the first and second

[1] The observations were made upon eggs taken from a tray of which about 90% of the remaining eggs was successfully hatched.

furrows to the *niveau* of the nuclei, that the blastomeres are of a more uniform texture, and that their nuclei are larger, more regular in outline, and more clearly differentiated in structure.

Variations of the eight-cell stage are shown in the accompanying figure, Fig. 4, the meridional form, *A* and Pl. III, Fig. 39, is not uncommon ; more usual variations are *C* and *D*, less common are *B*, *E* and *F*, and forms, as *G* and *H*, showing an irregular horizontal cleavage are the rarest, occurring in from 4–6% of the specimens of this stage which the writer has examined. A wide range of variation in the cleavage of this particular form, in view of its supposed affinities is naturally suggestive, although logically perhaps, of little morphological importance: for cleavage changes have been recorded in Petromyzon,[1] Teleosts[2] and amphibians,[3] and are, as yet, of doubtful significance, in cases referable to mechanical causes, alterations of temperature or of water density.

In the case of the Sturgeon, however, it should be stated that the variations in cleavage occurred in a general rate of proportion among the normal eggs of the hatching trays, and on this account appear to be worthy of especial interest. It would certainly appear evident that a variation had occurred in the amount of the yolk material, in cases sufficiently great to permit the third cleavage plane to become horizontal. This peculiarity of the Sturgeon egg will later be referred to, in the discussion of the relationships of Ganoids, as suggesting a tendency toward the evolution of a more perfect holoblastic condition. In the Gar-pike similar variation of cleavage does not occur ; of all the eggs examined — to the number of several hundred — no widely-marked differences from the conditions figured in Pl. I, Fig. 4, have been recorded.

The variations (Figs. *A–F*) are disposed symmetrically with reference to the first plane of cleavage (which passes from

[1] McClure, ('93) *Zool. Anz.*, XVI, p. 429.

[2] Ryder, H. V. Wilson, Agassiz and Whitman, and others.

[3] Rauber, Morph. Jahrbuch, 1883; Jordan, J. of Morph., 1893; v. Ebner, Festschrift f. A. Rollett, Jena, 1893; the results of experimental studies of Roux, Morgan, and Hertwig.

right to left in all figures), and are in this respect sufficiently noteworthy to suggest that the first cleavage plane in Sturgeon as in amphibian (as Roux[1] recently appears to have reconfirmed) determines the sagittal plane of the embryo.

Fourth cleavage is again vertical, and the 16-cell stage is not unlike that of Lepidosteus. According to Salensky there occurs in this stage in *ruthenus* horizontal cleavage; but on account of the many variations which probably occur here[2] as well as in *sturio*, the writer is still inclined to believe that the normal fourth cleavage[3] in the sterlet may prove to be vertical. In *sturio* at this stage the four central blastomeres, Pl. III, Fig. 40, are irregular in outline, often projecting above the surface, and are usually separated by well-marked fissures: they are still, however, connected with the underlying layer of the germ disc as in the earlier stage. Wide variation in the 16-cell stage is to be noted in the size and shape of the four central blastomeres, in the area and distinctness of the polar pigmentation, and in the degree of obliquity of the fourth cleavage furrow, the latter often tending to become meridional, and in cases equatorial. On the lower pole of the egg the first and second cleavage furrows have by this time intersected.

In the stage of *fifth segmentation* the writer's material is deficient.

The *sixth cleavage* is represented in Pl. III, Fig. 41, and may be compared with the corresponding stage of Lepidosteus in Pl. I, Fig. 8. Its lower pole is shown in Pl. III, Fig. 42. Cell division has by this time turned the blastodisc into a cap of cells of irregular size and outline; horizontal cleavages have occurred, notably in the region of the animal pole, but these cells have not been caused to be lifted above the surface of the egg; meridional cleavage has occurred in the marginal cells, but is expressed in an irregular manner, often by becoming oblique, separating only a corner of the marginal blastomeres;

[1] *Anat. Anz.*, 1894.

[2] Thus Salensky states, "Quand la partie supérieure de l'œuf (germe) est divisée en *dix* parties — the italics are the present writer's — il apparaît dans le germe des sillons transversaux."

[3] The writer has been unable to compare the results of Peltsam (*Ref.* 20).

sometimes, as in the left of the figure, cleavages remaining meridional, seem unable to penetrate the pigmented zone of the yolk and thus for a time remain undifferentiated. The connection between yolk and cytoplasm which thus maintains seems accordingly equivalent to the condition of the marginal cells in a corresponding stage of Lepidosteus, whose significance has already been discussed (p. 19). In the surface view of the animal pole the pigmentation, in early stages so characteristic of the Sturgeon, is hardly visible, and the fissures between the blastomeres are greatly reduced. The lower half of the egg is traversed by the third cleavage furrow, which intersects the first and second furrows at moderate angles. There are thus, therefore, in the 64-cell stage but 6 cells visible on the lower hemisphere, a condition which differs somewhat from that of *ruthenus*, where, according to Salensky, "lorsque l'on peut distinguer douze segments dans la partie supérieure de l'œuf, sa partie inférieure n'en montre encore que 6." Nor is there to be seen in the yolk region the boldly rounded cell outlines which Salensky has figured, *Ref.* 29, Pl. XV, Fig. 10.

A stage about one hour later, Pl. III, Figs. 43 and 44, already exhibits a great advance in development. The entire light-colored pole is now composed of finely divided cells, even to the margin of the pigmented zone; at the animal pole the outlines of the segmentation cavity may be faintly determined through the fissures between the cells, and the surface of the yolk-half of the egg is now seen subdivided into many-sized polygonal cells. A cross section of this stage, Pl. IV, Fig. 59, and of the same stage at the side, Fig. 58, shows important differences from the corresponding stage of Lepidosteus (*cf.* Pl. II, Fig. 28); the blastoderm is growing within the limits of the egg's spherical curve, instead of on its surface by increment from below. It will be seen that the segmentation cavity is here separated from the surface by but a single layer of cells, instead of by the cell-mass of the Gar-pike; that it is of more definite size and shape, that its floor is now cellular, and not of the clearly marked, merocyte-bearing, Elasmobranch type of Lepidosteus.

Blastula.

In this *blastula* of Acipenser, however, the relations between yolk and blastomeres may best be understood by referring them to the conditions of the Gar-pike. The periblast-like floor of the segmentation cavity in the latter form has now become reduced to a few irregular yolk-bearing cells, which in number and position correspond closely with the merocytes of Pl. II, Fig. 28. A reduction in the amount of yolk material might clearly account for this change. As in Lepidosteus the lowermost cells of the blastoderm are derived from cells whose cytoplasm is connected with the yolk, and are budded off, as in Pl. II, Fig, 29, from central as well as from marginal regions. The cells connected with the yolk are seen undergoing nuclear divisions, and appear to represent in function the merocytes of Lepidosteus. The irregular polygonal cells of the yolk hemispheres are found to possess dividing nuclei close to their outer surface, but are altogether indistinguishable in sections. Naturally, however, the elaboration of the yolk material, although occurring on every side, is most actively carried on in the uppermost part in connection with the cell growth of the blastoderm.

In a slightly later blastula the increased size of the segmentation cavity and its more definite outline may be seen in surface view, Pl. III, Fig. 45, and in section, Pl. IV, Fig. 60 (with this *cf. Ref.* 29, Pl. XV, Fig. 11). By study of the nuclear figures of the cells roofing the segmentation cavity in the preceding stage, it is found that the present two-celled segmentation-roof has been the result of horizontal cell cleavage. The thickened sides of the cavity appear due to the normal increase of marginal cells together with additions from the underlying yolk cells. These in the floor of the segmentation cavity have now produced a double layer of cells, whose upper elements are in size, texture, and nuclear character not to be distinguished from those at the sides. The region, in other words, of the yolk cells is retreating centrad. The writer notes that his description of this stage of the blastula does not agree with that of a corresponding stage of the sterlet given by Salensky.

The latter author has figured an early blastula whose segmentation cavity has a roof three cells in thickness, the sides one cell in thickness, and a floor lacking in differentiated cells. Salensky, in addition, figures the yolk mass totally traversed by one cleavage plane and partially by a second.

In a slightly later stage the walls of the segmentation cavity have greatly thickened. The roof and sides of the cavity are now of 8–9 cells in thickness and its floor is of 4–5 tiers of cells which preserve their yolk-cell characters. Differentiated cells in the lower hemisphere of the egg are not to be distinguished. Pigment is plentifully scattered in all cell layers of the roof of the segmentation cavity.

A later blastula has been figured in surface view in Pl. III, Fig. 46, and in vertical section in Pl. IV, Fig. 61. It is comparable to the stage which Salensky has figured in Pl. XVI, Fig. 12, but differs from the latter notably. Thus its yolk mass is not as yet divided into cells, the segmentation cavity is larger, more definite in outline, its roof thinner at the animal pole, its floor, slightly concave, composed of definite and uniform cellular elements. The stage figured immediately precedes gastrulation, and it is doubtless on this account that a marked asymmetry maintains in the roof of the segmentation cavity : below the thicker side will occur the dorsal lip of the blastopore : outwardly this stage is of finely finished appearance, in contrast with that of Fig. 43; continued division has rendered the cells of the animal pole so minute that this region appears waxy ; the marginal pigmented zone has in addition lost its well marked appearance, and the cell outlines of the lower hemisphere are distinguishable but faintly. The pigment which now appears concentrated at the animal pole is in the region where the roof of the segmentation cavity is thinnest (*cf.* Pl. IV, Fig. 61).

Gastrula.

Gastrulation begins at a stage represented in Pl. III, Fig. 47, and in vertical section in Pl. IV, Fig. 62. Outwardly this stage is closely similar to that last figured; it possesses the dark pigmentation at the animal pole, the white zone of minute

unpigmented cells, and the lower hemisphere of dusky color and faint cell outlines. But in the later stage each of these characters has become different in details: the pigmented tract at the animal pole is of lighter color and of obscurer boundary; the lower margin of the white zone is now more distinctly drawn and at one side is already to be recognized as the dorsal lip of the blastopore; the lower hemisphere is darker in pigmentation and its marking of cell outlines is more obscure. The section shows but minor changes from the conditions of Pl. IV, Fig. 61. The roof and floor of the segmentation cavity are thicker in cell layers: the cavity itself has received an angular extension in the region above the dorsal lip. The latter is observed as a well marked notch between the small and regular cells of the upper hemisphere and the large and irregular cells of the region of the pigmented zone. These now, for the first time, are to be clearly distinguished, and are seen to be largest at the region of the lip and smallest at the opposite side of the embryo. It is to be noted that the outermost stratum of the cells of the upper hemisphere has now differentiated as the 'Deckschicht.' This has been figured by Salensky, although not mentioned in his text. Further differences from the conditions described in *ruthenus* are to be found in (I) the roof of the segmentation cavity; this in *ruthenus* is constituted mainly of a layer of cells like those of the yolk region (*cf. Ref.* 29, Pl. XVI, Fig. 13), (II) the shape of the blastopore, which in the sterlet is described as crescentic, (III) the character of the blastoporic "invagination," since in *ruthenus* the lip of the blastopore appears to be widely separated from the yolk cells, and (IV) the qualitative difference, as maintained by Salensky, between the cells of the upper and of the lower hemispheres. The present writer would here note that in his studies of *sturio,* he finds nothing to warrant the qualitative distinction that the Russian author has drawn; he was able to observe in the cells of the upper hemisphere neither *zone corticale* of the cytoplasm nor any indications of the "petites bosselures qui ressemblent à des pseudopodes lobés," and believes it probable that these were due to an imperfect method of preservation.

In Pl. III, Figs. 48 and 49, are figured two later gastrulas, and vertical sections of those stages are given in Pl. IV, Figs. 63 and 64. Outwardly these stages are conspicuous, on account of the sharp color contrast they present in their unpigmented and pigmented zones; the vegetative pole of the egg is seen to darken in color as it becomes reduced in outward size by the constricting blastopore. The uppermost part of the embryo is in these stages somewhat depressed, and is slightly darkened by pigment. In the earlier gastrula the dorsal lip of the blastopore is more sharply drawn, — shown in the left of the figure, — while in the later form the dorsal lip is often indicated by a slight nick-like indentation of the rim of the blastopore. The thickening of the dorsal lip in the median plane, which shortly appears, is the first indication of the embryo's axis. Examination of sections of these stages enables us to better understand the advances which have taken place in the development. In the earlier gastrula the blastopore's dorsal lip has separated from the yolk cells and has enclosed about 45° of the egg's circumference; it is thicker comparatively than that of Lepidosteus, and already includes the three germ layers which are seen to become confluent near the margin of the lip. Its exact mode of growth can of course only be determined experimentally, but from the blunted end of the coelenteron it appears that to some degree a growth of the entire lip has taken place. The segmentation cavity has greatly flattened, to a degree in fact which renders it difficult to be determined in the anterior region of the embryo; its floor has also extended and flattened, and its four or five layers of loosely associated cells are often seen clearly distinct in many regions from the yolk mass. As yet only a trace of the anterior lip of the blastopore has appeared; here the cells of the lower hemisphere are seen to increase in size and irregularity in the direction of the dorsal lip. In this region pigment occurs plentifully, and is present in notable quantity in the cells lining the coelenteron.

The older gastrula may be directly compared with that of Lepidosteus, Pl. II, Fig. 31. It presents a marked advance in the growth of the blastopore; its dorsal lip now surrounds a quadrant, and its ventral lip about 20° of the egg's circumfer-

ence. The segmentation cavity has acquired a pronounced deepening in the region of the end of coelenteron, but its anterior limits, as before, remain difficult to be determined; its roof is now greatly reduced in thickness, in a large part of its extent a single-celled layer; it is densely pigmented and its cell boundaries may be distinguished only with the greatest difficulty. The immediate rim of the blastopore is thickened, especially in the dorsal lip: here the thickened rim, by being pushed against the yolk cells, has acquired a notch immediately in front of it, which will later be discussed as the homologue of Kupffer's vesicle. Pigment again occurs in all invaginated regions.

Contrasted with Lepidosteus, this stage of the Sturgeon will at once be seen to present differences which appear interpretable as of specialized character; thus we may contrast:

	Lepidosteus.	*Acipenser.*
Gastrula.	Two-layered.	Three-layered.
Segmentation cavity.	Simple.	Broadly dilated near end of coelenteron, its anterior limit almost indiscernible.
Roof of cavity.	Thick, little pigmented.	Thin, single-celled for a large extent of its surface, heavily pigmented.
Walls of coelenteron.	Unpigmented.	Pigmented.
Rim of blastopore.	Thin, somewhat tapering distally.	Thick, blunted, thickest at dorsal lip, where an anterior notch (Kupffer's vesicle) is present.

A late gastrula, Pl. III, Fig. 50, shows the continued reduction of the blastopore, and the appearance in surface view of the embryo's axis. The region surrounding the blastopore appears slightly conical in its surface curvature, the opposite end of the egg flattened and finely pigmented. The light-colored outer layer and the blackened yolk plug of Sturgeon present a well known contrast to the pigment conditions of the amphibian gastrula. The embryo is in this stage but faintly discernible; it appears as

a somewhat opaque tract of cells, whose anterior end, broadening widely, represents the cephalic plate, and whose obscure dusky line, perpendicular to the rim of the blastopore, is the *anlage* of the hinder neural axis. The blastopore is now circular; it is only at a stage represented in Fig. 49 that any indication of an indented rim has occasionally been found. In a slightly later stage are to be noted changes in the shape and roof of the segmentation cavity, in the growth of the dorsal lip and the rim of the blastopore, and in the coelenteron in its dorsal region. The dilated region of the segmentation cavity has become deeper, its anterior and posterior margins closer together and more sharply marked. The roof of the dilated area has thickened and is irregularly pigmented. While the ventral lip of the blastopore has remained as in the preceding stage, the dorsal lip, Pl. IV, Fig. 65, has increased greatly in length, now enclosing about 130° of the egg's circumference; in thickness, however, it has remained as in the last figure, except at the blastopore's rim. In this entire region an increase in cell material has become marked, least noticeable at the ventral and greatest at the margin of the dorsal lip. Pressure against the yolk plug seems here to have been reasonably the cause of the inflected rim. The coelenteron below the dorsal lip of the blastopore is notably deeper (ecto-entad) than in the former stage, its fundus is more broadly rounded, and its recessus immediately below the dorsal lip, *i.e.*, Kupffer's vesicle, is deeper and sharply trench-like. In the figure the limits of the germ layers are shown; the inner layer is here thin, but in the axial line becomes the thickened *anlage* of the notochord. The thickening of the outer layer which caused the appearance of the embryo is found to be exceedingly slight and can only be seen satisfactorily in transverse sections. The Deckschicht, which was earlier noted, cannot be satisfactorily distinguished in this stage.

Further growth of the embryo is illustrated in Pl. III, Fig. 51. Here the cephalic region is seen to have spread out, ovate in outline, as if flattened upon the rounded surface of the egg. The spinal axis is prominently marked, and at its hinder end communicates with coelenteron; a small light-colored band

bridging its hinder margins forms an imperfect roof for the neurenteric canal. The elliptical margin of the blastopore appears thick and opaque (Pl. IV, Fig. 66). From it, passing forward and slightly diverging, terminating near the hinder region of the head, are the pronephric ducts. The closure of the blastopore and the detailed establishment of the neurenteric canal may at this point be most conveniently understood. A condition later than that of Pl. IV, Fig. 64, is given in the same plate, Fig. 65: it illustrates the continued reduction in diameter of the yolk plug and its great increase in (ecto-entad) length: the inflected rim of the blastopore is now exceedingly deep, the recessus (Kupffer's vesicle) under the dorsal lip, prominent, deep but somewhat rounded: a general growth in extent of the entire outer wall of the coelenteron appears to have taken place since its distance from the yolk is seen to have greatly increased. At a subsequent stage, Fig. 66, when the blastopore has become reduced to but half the diameter of that last figured, the yolk plug loses its distinct cylindrical character: it comes to conform to every irregularity of the thickened blastopore. Further reduction of the diameter of the yolk plug takes place regularly: in Fig. 67, it has become greatly narrowed, and as the section is slightly oblique its connection with the neural canal may be seen: it still touches the surface at a narrowed point. An outward view of these conditions is to be seen in Pl. III, Fig. 53: the deepest point of the indentation between the tail folds is the entrance of the neurenteric canal; immediately below it, at the darkly shaded point, is the disappearing remnant of the yolk plug. In this figure may in addition be seen the hinder limits of the parietal zone, the outline of the neural axis, the undifferentiated tissue of the primitive segments, and at the embryo's extreme margin the hinder part of the pronephric ducts: the tail folds are greatly flattened, but at the hindermost margin are slightly raised above the surface. A final stage in the fate of the blastopore is to be seen in Fig. 54: the tail folds have fused in the median line, leaving a slit-like opening into the neurenteric canal to be seen at the surface. In sections, Pl. IV, Fig. 68, and Fig. 69, it seems evident that the lower part,

if not a large part, of blastopore is retained as the hindermost portion of the neurenteric canal, its greatly diminished yolk contents disappearing. In the surface view, Pl. III, Fig. 54, may be seen prominently the neural canal, primitive segments, and, now depressed and passing under the flattened tail mass, the pronephric ducts.

The origin and fate of the vesicle of Kupffer appears closely connected with the closing blastopore. Within the entire circle of the blastopore's rim occurs the trench which in section has been noted in the different stages of gastrulation. When seen in vertical section it appears under the dorsal lip as the deep recessus which the present writer regards as Kupffer's vesicle. In Sturgeon the fate of this structure (which may be followed in the sections, Pl. IV, Figs. 64, 65, 66, 68, 69) shows conclusively that it can be regarded as but a growth adaptation of the gastrula, a condition due to an extreme thickening of Randwulst. It is most marked in the median line in the tail region, since it is here that the embryo has acquired a keel-like thickening which exerts a mechanical influence in deepening the Randwulst and causing the underlying cavity to become enlarged. In the Teleost where the concentration of the embryo in the median plane is extremely marked, Kupffer's vesicle may well represent an expression of its mode of growth : the germ ring is clearly the rim of the blastopore — or more accurately perhaps the circumcrescence margin — of Acipenser. This interpretation of the vesicle, the writer believes, would adequately explain its peculiar conditions in *Salmo* or *Esox:* in *Salmo fario* he has examined the vesicle and verifies the observation of Henneguy as to its possessing a flooring of entoderm cells: this layer, loosely separate from the undifferentiated cells in the anterior region of the tail mass, might, the present writer believes, readily remain in contact with the periblast below, as the mechanical growth change caused the vesicle to appear.

The outline of the early development of the Sturgeon might now best be completed by a discussion of the differentiation of the germ layers.

The origin of the outer layer has already been traced (pp.

37, 38). In Pl. III, Fig. 51, the head outline is entirely an epiblastic thickening: anteriorly it is a pad of almost uniform thickness ; caudad the thickness of epiblast tapers away, but at the rim of the blastopore suddenly increases, dipping down into the undifferentiated tissue. These characters of the epiblast may be well seen in the (nearly) sagittal section of Pl. IV, Fig. 66.

The central nervous system is earliest developed at its extreme ends, brain and neurenteric canal. Between the condition figured in Pl. III, Fig. 51, and in section Pl. IV, Fig. 66, and that of the stage of Pl. III, Fig. 52, and of the sagittal section Pl. IV, Fig. 71, — slightly earlier — notable changes have occurred: there has been a concentration of formative epiblast in the brain region ; its anterior end has dipped deeply down, and has acquired a lumen, whether originally by cell dissociation or by process of invagination the writer has not been able to determine. He is certain, however, that in the initiatory process the cell thickening was deep and sharply marked in the median line. In the section given it is of especial interest to note the point of union, x, of the front end of the brain with the formative epiblast: it is situated at a remarkable distance tailward. A cross section at this point is figured in Pl. IV, Fig. 72. It is evident from the longitudinal section that the extension of the brain forward has been caused by the increase in size of its sides and floor. The width and flatness of the ventricle should be noted. By study of serial sections tailward of this stage it is found that the neural canal becomes thicker (shallower) and narrower: there occurs no evidence that the central canal has been formed by cell dissociation ; the formative epiblast rounds abruptly into it, and it appears broadly trench-like: it is roofed, however, dorsally by a strip of cells which appear in contact with both Deckschicht and marginal cells of formative epiblast (Pl. IV, Fig. 73).

In the present paper it is not intended to discuss the studies of Kupffer on the morphology of the head of vertebrates. His admirable description of the later development (beginning with the 45th hour) of the brain of Acipenser has been followed by

the present writer and his observations generally confirmed. His studies of stages earlier than Kupffer had secured do not, however, permit him to believe with the German author, "dass sich beim Stör das ursprüngliche Vorderende des Neuralrohres mit Sicherheit hat feststellen lassen," although not doubting that in the *Lobus olfactorius impar* exists the last connection between brain and epiblast. The earliest Vorderende of the neural canal which the writer has observed occurred in a stage about twelve hours earlier in development than that figured by Kupffer. It has already been noted in Pl. IV, Fig. 71. Between this stage and that of Kupffer it has been found that connection exists with the epiblast in front of this point, which corresponds in position with that of Ep^2 of Kupffer's Fig. 13. Sometimes in fact the entire roof of the brain in this region can hardly be regarded as separate from the epiblast : and even a short time before the Lobus comes to be distinctly marked, the anterior region of the brain is for a broad extent (x–x) firmly fused with the epiblast (Pl. IV, Fig. 74): that this fusion together with that of the later occurring Lobus is of secondary origin, the present writer is satisfactorily convinced. A discussion of the mode of origin of the hypophysis is reserved for future publication. It may in summary be said that the mode of development of Acipenser appears peculiarly specialized, and that many of its essential features in earlier stages seem entirely due to its flattened conditions of growth.

The latest stage in the development of the central nervous system to be noted in the present paper is that figured in Pl. IV, Fig. 52, several hours earlier in development than that of Kupffer's earliest stage (Fig. 1). The rather prominent outline of brain and optic vesicles may in sections be shown to be partly due to pigment contained in the cells of the floors of these cavities. The fore-brain is seen continued far forward, the mid-brain is proportionately large, and the hind-brain is as yet unwidened. The tapering tip of the fore-brain indicates indistinctly the *Lobus olfactorius impar*, which is here the last point of connection between brain and epiblast. The light-colored margins of the central canal are now clearly marked ; a

surrounding and indistinct zone of dusky color is mainly mesoblastic, while outermost the dark-colored parietal region indicates the general boundary of the fore-gut : in its foremost margin is the *anlage* of the heart. Pronephric ducts appear at the sides of the neural axis, terminating in a somewhat obscure way in the neighborhood of the hind-brain. As yet traces of neither gill slits nor auditory vesicles have appeared.

The *inner germ layer* may next be considered. In the stage of Pl. IV, Fig. 64, the entoderm may be traced from within the rim of the blastopore, lining its margins, continuous with the cells of the surface of the yolk mass. Upon the closure of the blastopore, Figs. 66, 67, 70, the entoderm of the outer wall of the coelenteron is a layer of cells distinctly separate from outer layers : at its lateral margins it gradually becomes continuous with the cell layer of the yolk which constitutes accordingly the inner wall of the gut. The yolk is therefore to be interpreted as identical in its relations with that of Elasmobranch, Teleost, or urodele, and is not to be compared to that of Ichthyophis, as suggested by Ryder, *Ref.* 22. At this stage the extent of the gut is to be seen in the sagittal and cross sections above referred to. The outer limit of the parietal zone of Figs. 50, 51 marks in general the boundary of the gut. The notochord arises in the normal manner, a rod-like thickening of the hypoblast in the line of the embryo's axis. Beginning at the rim of the blastopore, thick and wide, as it extends forward it diminishes in size, Fig. 70, and is finally indistinguishable in the region of the hind-brain. The writer can find nothing in the mode of origin of the notochord to suggest the mesoblastic derivation maintained by Salensky.

The *mesoblast* in Acipenser was regarded by Salensky as derived from the entoderm in as early a stage as that figured in Pl. IV, Fig. 62. Here the entire non-entodermic portion of the rim of the blastopore seems to be identified by the Russian author as 'mesoderm,' the entoderm extending around the yolk mass but reaching no farther than the ring-like end of the coelenteron. This 'mesoderm' was accordingly described as growing

"aux dépens des cellules de l'entoderme, qui est tout au moins le principal facteur dans sa formation, et non pas aux dépens du bourrelet marginal." Of the entoderm cells at the margin of the ingrowing coelenteron the more superficial are in their turn continually becoming mesodermic; "après s'être multipliées, elles prennent des caractères très semblables à ceux des cellules du bourrelet marginal, et de cette façon, tant que la cavité digestive primitive s'étend, le mésoderme s'accroît de bas en haut aux dépens de l'entoderme." From sections of well preserved material a quite different interpretation of the origin and growth of the mesoblast is to be obtained. In Pl. IV, Fig. 63, it will be seen that in a very early stage of gastrulation the lip of the blastopore is already divided into its three germ layers; the outer layer thickens and is deeply inflected at the lip of the blastopore, to a point where it becomes connected with the middle and inner layers: here the inner layer is thinnest, thence it widens, but near the end of the coelenteron, merging with the yolk-laden cells, its boundary is no longer to be traced: it is now indistinguishable from the middle layer which from the lip of the blastopore up to this point has remained distinct. At a later stage, Fig. 64, the inner layer of both dorsal and ventral lips is seen to be continuous with the large entoderm cells of the yolk mass: at the Randwulst the three layers merge; in the region ectad of the end of the coelenteron the mesoderm passes into the yolk mass. In Figs. 65, 66, the germ layers are seen more widely separated, and their confluence in the Randwulst is more prominent. In the section of the greatly reduced blastopore, Fig. 67, the middle layer in this region is to be clearly seen separate from the inner layer and confluent with the yolk cell mass. In transverse sections of this stage, Fig. 70, the presence of gastral mesoderm may be established for a short distance in front of the blastopore (equivalent to about one-sixth of the length of the embryo of Pl. III, Fig. 51); further forward than this the layer of mesoblast decreases notably in thickness, but always maintains its connection laterally with the yolk mass.

The mode of early development of the middle germ layer of

Acipenser presents a suggestive contrast with that of Lepidosteus (p. 24).

	Lepidosteus.	*Acipenser.*
Middle layer appears	Late: about the time of the closure of the blastopore.	Early: shortly after the appearance of the ventral lip of the blastopore.
Its early character	A discoidal cell mass, almost symmetrical, its elements alone in contact with inner and outer germ layers at its center, the blastopore.	A ring-like cell mass, notably asymmetrical, confluent with inner and outer layers at the rim of the blastopore, and confluent at its outer (peripheral) margin with the yolk mass.
Its mode of growth	At first peristomal; becomes greatly thinned: at its periphery single-celled, mesenchymatous. Gastral mesoderm early apparent in hinder region of embryo's axis thickened notably near the median plane (in stages of Pl. I, Figs. 18, 19), connected with entoderm in the hinder region of the embryo's axis.	Notably peristomal, a layer of almost uniform thickness from rim of blastopore to undifferentiated yolk tissue. At stage of Pl. IV, Fig. 66, its thickness has become reduced in its peripheral region. Gastral mesoblast differs little in thickness from neighboring peristomal mesoblast: the region of its connection with entoderm is restricted to that of the hinder one-sixth (about) of the embryo's axis.

The earlier appearance of the mesoblast in Acipenser seems to the writer to be probably due to the smaller amount of food yolk in this form. The difference, however, in the plane of early mesoblastic growth is hardly to be ascribed to this cause alone: the extremely compressed or rather horizontal mode of growth of the Sturgeon would, however, seem adequate to explain this character. The mesoblast has a normal separate growth till in the region of the end of the coelenteron: a possible mechanical cause may here prevent its separate extension; and a similar reason would account for the uniform thickness of the layer of gastral mesoblast.

IV. General Comparison of the Early Stages of Garpike and Sturgeon, and Conclusions.

In the foregoing paper the similarity in the mode of development of these kindred Ganoids has been noted in some detail from stage to stage. There have thus been compared their rate of development (p. 12), the positions assumed during the growth of the embryo (p. 13), the size, adhesiveness, mode of pigmentation and deposition of the egg (p. 14), the cleavage (pp. 30–34), gastrulation (p. 39), and the origin of the mesoderm (p. 47). There yet remains to be given a summary of the results of the present writer which shall contrast the general processes of growth, *i.e.*, segmentation, the development of the primary germ layers and the establishment of the embryo's external form.

The segmentation of Lepidosteus appears clearly of a more meroblastic character than hitherto described: in none of the writer's material have cleavage planes been observed traversing even superficially the yolk pole of the egg. The germ substance is sharply separate from the yolk; its specific gravity appears notably lighter, and at the animal pole it often projects above the surface curvature of the yolk mass. Cleavage is early expressed in regular planes, giving the blastomeres almost conventional outlines; it is only after the fourth cleavage that variations are observed.

In Acipenser, on the other hand, segmentation is holoblastic, although the furrows traversing the yolk region are of an exceedingly superficial character.[1] The consistency of the substance of the germ is more nearly that of the yolk, and the surface curvature of the early blastomeres corresponds with that of the egg. In general cleavage planes are deepest in the region of the animal pole, becoming more and more superficial as they pass around the egg to its lowermost point: they agree in general direction with those of Lepidosteus but are irregular in their mode of occurrence from the second plane onward.

[1] As noted by Salensky and others.

In pigmentation the eggs of Acipenser present the strongest contrast to those of the Gar-pike.

The stages of late segmentation may thus be contrasted. In Lepidosteus the division of the blastomeres results in a cap of cells of irregular sizes : this appears as a distinct prominence of the egg surface: below it a smooth surfaced periblast-like layer contributes at irregular points to the growth of the blastoderm, — a condition maintaining till the time of gastrulation. The cell cap of Acipenser is far more closely continuous with the yolk ; cells large and irregular in size are readily distinguished in an intermediate zone between blastoderm and yolk: of this zone the number of tiers of cells agrees in a general way with that of the merocytes in corresponding stages of Lepidosteus.

The general differences in segmentation appear due to either a greater or decreased amount of the egg's reserve of yolk material. But, everything considered, it seems to the present writer more probable that the mode of segmentation of Acipenser, although holoblastic, is more readily to be derived from that of Lepidosteus than *vice versa*. The reasons for this view of the relationship of the Sturgeon are based: (1) on the knowledge of its descent as afforded by palaeontology,[1] (2) on the curiously superficial character of its total segmentation, (3) on its marked irregularity of cleavage, — a character by no means conclusive in itself, but important in connection with (1) and (2). If the evidence be ultimately accepted as to the derivation of the Sturgeon and of its mode of segmentation, it is obvious that the results of Beard in his paper on the Interrelationships of the Ichthyopsida would be seriously disarranged.

In the development of the primary germ layers the differences of these forms appear in like manner due to the alteration in quantity of yolk-material. In Lepidosteus the clearly marked surface between cell cap and yolk maintains until the time of gastrulation ; in Acipenser a transitional zone of irregular yolk cells exists from an early stage. In one form the cap of cells is of irregular character, in the other it is reduced to a

[1] *Cf.* esp. A. Smith Woodward, On the Palaeontology of Sturgeons, *Pro. Geol. Ass.*, Vol. IX, Nos. 1 and 2.

definite layer. In Gar-pike the segmentation cavity, when definitely formed, undergoes the smaller changes in extent and outline, and its roof remains of more uniform thickness. The alterations which these conditions have been seen (p. 38) to undergo in the Sturgeon seem of more specialized character. Early gastrulation is closely comparable in both forms: that of Lepidosteus, although hampered in development by the presence of a greater amount of yolk material, seems more generalized: in this form for example, as previously noted, p. 22, a two-layered condition maintains until the time of the blastopore's closure, the inner and outer layer of the dorsal lip are essentially identical in structure, and as in the elasmobranchian gastrula there occurs no growth modification of the rim of the blastopore to give rise to a Kupffer's vesicle. It also approaches the type of the Elasmobranch in the character of the superficial cell stratum, which it early develops, but diverges more widely than Acipenser in the matter of neurenteric canal. Of the development of the middle germ layer, p. 46, the conditions that have been noted in Acipenser seem more specialized than those of Lepidosteus; thus the early peristomal mesoderm in the latter form is independent in its distal margin from either outer and inner layers, and presents a strong contrast to the restricted and abbreviated layer of Sturgeon. The gastral mesoderm, moreover, although similar in both forms in its apparent mode of origin, suffers in Sturgeon a flattened growth which can only be regarded as of specialized character.

The growth processes establishing the outward form of the embryo furnish perhaps the strongest ground of contrast in the early development of Gar-pike and Sturgeon: in the former the form outline of the embryo is constricted off from the yolk mass; in the latter the embryo, in a mode of growth greatly flattened, continues up to a late stage to surround the yolk, and to preserve outwardly the spherical curvature of the egg.

The greatly flattened growth conditions of the Sturgeon embryo are certainly unique among vertebrates; and among fishes — using the word in its broadest sense, — they may reasonably be regarded as peculiarly aberrant. The mode of establishment of the early external form of the Gar-pike is not

unlike that of Teleost, and is not strikingly different from that of Elasmobranch, or even of urodele ; and in view of the undoubted kinship of Lepidosteus and Acipenser it seems impossible to regard the peculiar growth of the latter as essentially the more primitive and generalized. What the conditions have been that have caused the flattened form-growth of the Sturgeon is not easily to be established, but the greatly enlarged outline of the embryo's head indicates the stages when they are most prominently marked. The brain region in these stages does not appear, accordingly, particularly suited for the study of conditions which are to be regarded as undoubtedly primitive: secondary characters, *e.g.*, fusions of epiblast with the roof of the fore-brain, or with the entoblast of the fore-gut, appear to occur most confusingly.

In concluding the above summary of the essential characters of the early development of Lepidosteus and Acipenser an especially noteworthy result should be emphasized — that Acipenser in its main development features seems the more specialized type, but that it suggests in essential regards its descent from a form not unlike Lepidosteus. The evidence thus furnished seems to add the needed confirmation to the results of Smith Woodward and Traquair, who have derived the Sturgeons from a Palaeoniscoid stem, and recognized in this a close kinship (*e.g.*, through Dictyopyge and Catopterus) with the scaly Ganoids. The Sturgeons (Smith Woodward) 'descending from this stem have evolved an increased size and have degenerated or specialized in conditions of exoskeleton.'

As to the evidence of the kinships of Ganoids afforded by study of their early development: —

Both A. Agassiz and Balfour and Parker have already emphasized the nearness developmentally of Gar-pike and Teleost. *Cf. Ref.* 5, p. 430, (1)–(5).

In addition to the teleostean characters previously noted the present writer would add : —

(1) Similarity in the mode of the first four cleavages.

(2) Kindred relation of merocyte zone of germ disc to periblast, suggesting that in the latter a thinning away of the

merocyte zone has occurred in the middle of the floor of the segmentation cavity.

(3) Comparison of the gastrulas (*cf.* p. 39) ; the rim of the blastopore becomes the germ ring ; the "ventral mesoderm" of H. V. Wilson, the primitive hypoblast of the blastopore's ventral lip : of both lips the marginal fusion with periblast-yolk is in Teleost obliterated, — probably on account of the enlarged size of the yolk, and the more perfect relations of periblast to embryo. The interpretation of the Teleost gastrula of Ziegler becomes accordingly slightly modified: coelenteron extends under the rim of the blastopore from the free end of the "ventral mesoderm" to that of the "primitive hypoblast" of H. V. Wilson.

(4) The presence of Kupffer's vesicle in Acipenser, v. p. 42.

But it is especially significant that the nearness of Ganoids to Elasmobranchs in their adult conditions is also to be emphasized in their early development. In Lepidosteus the shark-like characters in partial segmentation, the formation of merocytes, the mode of origin of the germ layers, have already been noted. Of Acipenser the conditions, not widely different, have retained in addition a neurenteric canal. In this comparison it is now evident that difference in size of egg or in the character of its membranes cannot be adduced as an insurmountable objection. Laemargus has shown that an egg capsule is not infallibly an elasmobranchian feature ; and by an analogy the difficulty in accounting for the increase or decrease in the number of eggs (as urged for example by Beard [1]) seems practically solved in the case of Bdellostoma and Petromyzon, forms universally regarded as of close genetic kinship. In Bdellostoma [2] the few and large encapsuled eggs, moreover, are probably extremely meroblastic.

The nearing of the phyla of Ganoids and Elasmobranchs on the evidence of early developmental characters seems worthy of especial consideration. Morphology would long since have established the stem of the sharks as most primitive and ancestral of existing gnathostomes, had not marked differences been

[1] *Anat. Anz.*, 1890, pp. 146–159 and 179–188.

[2] Ayers, 1894. Lectures of Woods Holl Marine Biological Laboratory.

adduced in ontogeny. Where characters of primitive holoblastism were sought, a condition extremely meroblastic was found; and until the question of the possibility of a gain or loss in food yolk should be decided, the segmenting stages of a Ganoid made its ancestry appear more primitive than that of existing sharks. What were the developmental conditions of Cladoselachid or Pleuracanthid can never be understood, but so closely did many palaeozoic sharks approach existing genera in the smallest details of exoskeleton that their development could with but little probability have been widely different. But it would now seem evident that the Ganoid which retains in the main the skeletal and exoskeletal features of the palaeozoic types, possesses as well characters in development which are suggestively elasmobranchian. And on the other hand a Ganoid (Sturgeon) which has widely diverged from its palaeozoic kindred is now found to represent a type of development which is in the main to be referred to the simpler characters of the more ancient Gar-pike, — but is at the same time more nearly holoblastic. That total cleavage is in this case of secondary nature seems a not illogical conclusion, and should certainly strengthen the position of Rabl in the question of the loss of food yolk.

The study of the early development of Lepidosteus and Acipenser leads to the conclusion that the ancestors of the Ganoids were meroblastic, rather than holoblastic, and that their kinship, as far as our present knowledge can decide, was most nearly with the Elasmobranchs.

Laboratory of the Department of Biology,
Columbia College, August 25, 1894.

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EXPLANATION OF PLATE I.

The Early Development of *Lepidosteus*.

All figures have been drawn from material fixed in alcoholic picro-sulphuric acid: the egg membranes of the early stages (Figs. 1–7), not separated readily by needles, have been removed after a brief immersion in 20% Javelle water. × about 20.

FIG. 1. Egg immediately before the appearance of the first cleavage furrow. ¾ hour after fertilization.

FIG. 2. First cleavage: it is noted as a trench-like furrow, extending no further marginally than the limit of the germ disc. 1 hour.

FIG. 3. Second cleavage: limited as before to the germ disc, whose margins now merge slightly into the yolk mass. 2 hours.

FIG. 4. Third cleavage: similar in marginal extension to first and second. 3 hours.

FIG. 5. Third cleavage: seen in side view. The margin of the germ disc is indicated.

FIG. 6. Fourth cleavage: similar in its limits to the third cleavage. 3¾ hours.

FIG. 7. Fifth cleavage. 4½ hours.

FIG. 8. Sixth cleavage. Horizontal cell division occurs for the first time: it is to be noted irregularly within the ring of marginal cells. 5½ hours.

FIG. 9. Seventh cleavage. Meridional furrows, which in the preceding stage have attained almost the equatorial region of the egg, appear in this stage greatly reduced. 6¾ hours.

FIG. 10. Eighth (?) cleavage. In this stage an irregular marginal depression and an elevated mass of cells of the animal pole are to be observed. 8 hours.

FIG. 11. Late segmentation, showing marginal groove and flattened cell cap. 20 hours.

FIG. 12. Late segmentation. The thickened cell cap is shown in a stage in which it is coming to overspread the marginal groove. 25 hours.

FIG. 13. Early gastrulation. 32 hours.

FIG. 14. Gastrulation. In front of the blastoderm's marginal indentation the embryonic thickening is to be faintly seen. 38 hours.

FIG. 15. Late gastrulation, showing circular blastopore. 44 hours.

FIG. 16. Late gastrulation, a stage slightly older than that of Fig. 15. An indented blastopore rim is here exceptionally present.

FIG. 17. Early embryo, showing the flattened vascular area in front of the head eminence, and the closed blastopore. 60 hours.

FIG. 18. Side view of the embryo of 60 hours.

FIG. 19. Embryo, showing an early stage of the establishment of its external form. 80 hours.

FIG. 20. Embryo of 90 hours.

EXPLANATION OF PLATE II.

The Early Development of *Lepidosteus*.

FIG. 21. Vertical section of egg of about 1 hour after fertilization, showing the first division of the nucleus. × 20.

FIG. 22. Vertical section of stage of 2 blastomeres. × 20.

FIG. 23. Horizontal section through the nuclei of 4-cell stage. × 20.

FIG. 24. Similar section of 8-cell stage. × 25.

FIG. 25. Similar section of 16-cell stage. × 25.

FIG. 26. Vertical section of 16-cell stage. × 20.

FIG. 27. Vertical section of stage of sixth cleavage, showing yolk nuclei, *m*. × 20.

FIG. 28. Vertical section of stage of 20 hours, showing zone of yolk nuclei. × about 55.

FIG. 29. Vertical section of stage of 25 hours. × about 65.

FIG. 30. Vertical (sagittal) section of stage of 32 hours. × about 45.

FIG. 31. Vertical (sagittal) section of stage of 40 hours. Coelenteron, *c*; dorsal lip of blastopore, *d*; segmentation cavity, *s*; innermost limit of coelenteron, *x*, *x*. × 30.

FIG. 32. Vertical (sagittal) section of region of blastopore in stage of 42 hours. Coelenteron, *c*; dorsal lip of blastopore, *dl*; epidermic stratum of outer germ layer, *e*; inner germ layer, *i*; middle, *m*; outer, *o*; yolk, *y*. × about 100.

FIG. 33. Transverse section of hinder trunk region of embryo of 80 hours (= 7 primitive segments). Chorda, *c*; epidermic stratum of outer germ layer, *e*; middle germ layer, *m*, inner, *i*; neuron, *n*.

FIG. 34. Sagittal section of embryo of 44 hours. Location of closed blastopore, *bp*; epidermic stratum of outer germ layer, *e*; inner layer, *i*; middle layer, *m*; outer layer, *o*; here of head region; yolk, *y*. × 75.

The material of the above sections was fixed in alcoholic picro-sulphuric acid; Figs. 21, 22, 23, 35, Delafield's haematoxylin, 24–29, concentrated alcoholic picric and acid fuchsin, the remainder, haemacalcium. Egg membranes when present in preparations have not been represented. With few exceptions the figures are from camera drawings.


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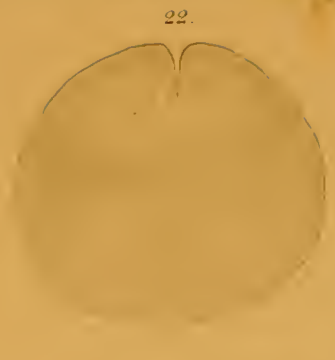
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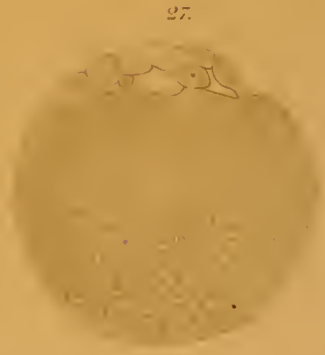
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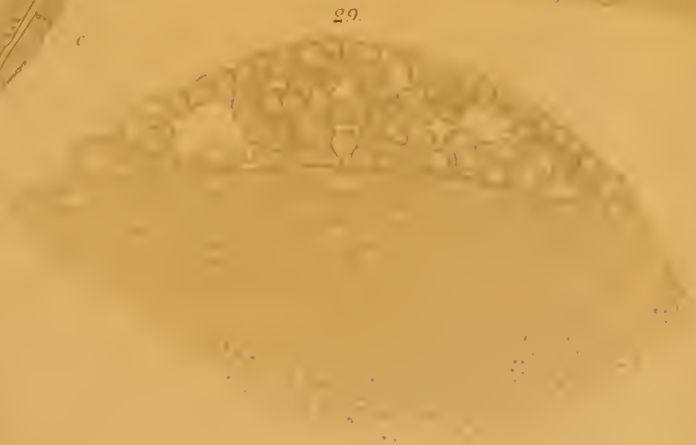
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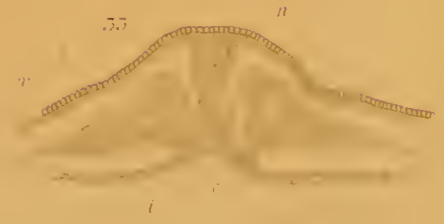
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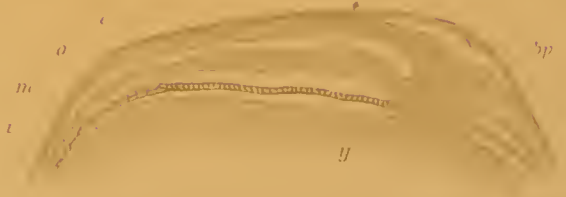
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EXPLANATION OF PLATE III.

The Early Development of *Acipenser sturio.*

Egg membranes of the stage of Fig. 43 and later, were removed with needles before fixation; those of the earlier stages were separated in dilute Javelle water. × about 23.

FIG. 35. Egg immediately prior to fertilization.

FIG. 36. First cleavage, 1 hour after fertilization.

FIG. 37. 1½ hours.

FIG. 38. Third cleavage, of normal type. 2½ hours.

FIG. 39. Third cleavage, of meridional type, seen in side view. The second furrow is to be observed traversing the yolk half of the egg.

FIG. 40. Fourth cleavage, of extremely regular type. 3¼ hours.

FIG. 41. Sixth cleavage. 4 hours.

FIG. 42. Sixth cleavage. Lower pole of egg.

FIG. 43. Late segmentation. 6 hours.

FIG. 44. View of animal pole of stage of 6 hours.

FIG. 45. Blastula. View of animal pole, showing through its transparent roof the outline of the segmentation cavity. 8½ hours.

FIG. 46. Blastula in latest stage. 16 hours.

FIG. 47. Early gastrula. The dorsal lip of the blastopore is seen at the left of the figure. 19½ hours.

FIG. 48. Gastrula, in side view, dorsal lip at the left. 26 hours.

FIG. 49. Gastrula, showing faintly marked indentation of the dorsal lip. 28½ hours.

FIG. 50. Late gastrula, indicating obscurely the outline of the embryo. 32 hours.

FIG. 51. Embryo of 43 hours. Eight primitive segments are present but are not distinguishable in surface view. In this stage are to be noted: the marked lateral expansion of the head region, the early neurenteric canal, the mode of closure of the blastopore — caused apparently by the more rapid growth of the ventral lip.

FIG. 52. Embryo of 46 hours: view of head and anterior trunk region. As yet the primitive segments — of which about 14 are now present — are not noticeable in surface view. The brain and optic vesicles are shown in an undifferentiated condition, their outlines prominently marked on account of pigmentation. The dark parietal zone of the embryo indicates the limit of the fore-gut. The region of the pronephric tubules is obscure.

FIG. 53. Embryo of 46 hours: view of tail region, showing the location of the neurenteric canal and of the almost closed blastopore.

FIG. 54. Embryo of 48 hours: view of tail region, showing the late appearance at the surface of the neurenteric canal. About 20 primitive segments are here present.


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EXPLANATION OF PLATE IV.

The Early Development of *Acipenser sturio.*

FIG. 55. Vertical section of egg immediately before first cleavage. × 22.

FIG. 56. Horizontal section (slightly oblique) of 4-cell stage, through the region of the nuclei. × 22.

FIG. 57. Horizontal section of 8-cell stage, through the region of the nuclei. An irregular cleavage is to be seen in the left side of the figure: the first cleavage plane of this and of the preceding figure passes right and left. × 25.

FIG. 58. Vertical section of 5-hour stage, showing cell outlines in the floor of the segmentation cavity. × 25.

FIG. 59. Vertical section of a stage of nearly the same age as that of Fig. 58, showing in greater detail the relation of the yolk cells to the mode of growth of the blastoderm. × about 65.

FIG. 60. Vertical section of 6-hour stage. × 25.

FIG. 61. Vertical (sagittal) section of stage, immediately prior to gastrulation. The posterior region of the embryo is at the right of the figure. × 25.

FIG. 62. Sagittal section of early gastrula (17 hours). The region of the dorsal lip is notably pigmented, and the outlines of the yolk cells in its vicinity may now be outlined. × 25.

FIG. 63. Sagittal section of gastrula of 26 hours, in which the ventral lip of the blastopore appears as but an indentation at the right side of the figure. The middle, inner, and outer germ layers are seen to be present in the dorsal lip. × 25.

FIG. 64. Vertical section near sagittal plane of gastrula of 29 hours. The inner, middle, and outer germ layers of the dorsal lip are lettered, *i*, *m*, *o*; the thinness of the inner layer is here accounted for in its proximity to the chorda; segmentation cavity is lettered *s*; coelenteron, *c*; and the vesicle of Kupffer, *k*. × 25.

FIG. 65. Section near the sagittal plane of region of the blastopore in stage of 32 hours. The structures of the dorsal lip are lettered as in preceding figure, and are seen readily comparable with those of the ventral lip. × about 65.

FIG. 66. Vertical (nearly sagittal) section of embryo of 43 hours. The thickening of the outer layer in the head region is lettered *h*, and although the yolk plug is now greatly constricted, the structures of dorsal and ventral lips and the enlarged coelenteron may readily be made out by comparison with Fig. 65. As yet the Deckschicht is not clearly defined and has been outlined in the figure.

FIG. 67. Transverse (slightly oblique) section through the region of the neurenteric canal of an embryo somewhat older than that of Fig. 66. Germ layers are indicated as before: neurenteric canal, *n*; yolk cells, *y*; yolk plug, *yp*, now disappearing; on either side of its position is the undifferentiated cell mass. × 45.

FIG. 68. Section almost sagittal of neurenteric canal of embryo of 46 hours. It is now seen definitely established, *n*; the site of the yolk plug is indicated as a slight diverticulum of its hinder wall, *yp*. × about 100.

FIG. 69. Sagittal section of neurenteric canal of embryo of 58 hours. It now opens widely into the gut, *n*, and its cellular wall is well established; the caudal

mass is seen at the left; at the right the notochord has separated from the inner layer; the epidermic stratum of the outer layer is now clearly marked, *e*. × about 100.

FIG. 70. Transverse section of the hinder trunk region of an embryo of 43 hours, showing the relation of the inner layer to the chorda and mesoblast.

FIG. 71. Sagittal section of anterior end of neuron of embryo of 11 primitive segments, slightly older than that of Fig. 66, showing the point of connection of the anterior end of the embryonic brain with the formative epiblast, *. The germ layers are lettered as before; the Deckschicht of the outer layer, *d*, is now clearly differentiated.

FIG. 72. Transverse section through the point * of the preceding figure. Lettering as before.

FIG. 73. Transverse section of region slightly posterior to that of Fig. 72.

FIG. 74. Sagittal section of anterior end of neuron of embryo of 46 hours. The lettering corresponds with that of Fig. 71. Between the limits *x*-*x* occur points of union between brain wall and formative epiblast, *o*; the anterior third of this region is broadly fused with the outer layer. Fusion also occurs in front of the embryonic brain between the formative epiblast and the cells of the inner layer, *i*.

Figures, with exception of Figs. 1, 2, 3, 46–48, are from camera drawings. Material was fixed either in picro-sulphuric or in corrosive glacial acetic. Haema-calcium and Delafield's haematoxylin were the staining agents.


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EMBRYOLOGY OF THE ISOPOD CRUSTACEA.

J. PLAYFAIR McMURRICH.

CONTENTS.

THE observations recorded in the following pages were commenced in the summer of 1890 at the Marine Biological Laboratory, Woods Holl, Mass. The object in view was to follow out for the Crustacea the cytogenetic development after the manner which has yielded such important results in the case of *Clepsine* (Whitman) and *Nereis* (Wilson), and after examining some early stages of several different species of Decapods, Isopods, and Amphipods, I finally chose for my purpose the ova of *Jaera marina* (Fabr.) Möbius, which seemed to present several advantages, notably in the early differentiation of the germ-layers. In addition, at that time the embryology of the Isopods had received, on the whole, less attention than it was entitled to, and considerable differences seemed to exist in the modes of segmentation of the forms which had come under ob-

servation. A preliminary notice of my observations upon this form was published in the *Zoologischer Anzeiger* in the summer of 1892, and seemed of sufficient interest to lead me to extend my studies to *Asellus communis*, Say, and later to *Porcellio scaber* and *Armadillidium vulgare*, the greater portion of the material being collected at Woods Holl. To my friend, Dr. W. M. Wheeler, I am indebted for a considerable quantity of material, which came originally, I believe, from Naples, and illustrates the development of *Cymothoa*, and to Dr. F. H. Herrick for material from *Ligia*, collected at Beaufort, N. C. In neither of these cases, however, were the earlier stages of segmentation represented, and I could make use of them only for the later stages of development.

In the present paper I do not propose to enter in detail into the organogeny of the group, my observations on this department of the subject not yet being sufficiently advanced. I shall have occasion, however, to refer to certain facts which have been made out concerning the development of some of the organs, and consequently shall divide the paper into four portions, the first of which will treat of certain phenomena connected with the impregnation and formation of the egg-membranes of *Jaera;* the second, of the segmentation and the development of the germ-layers; the third, of the later development of the mesoderm and endoderm; while in the fourth, some scattered notes concerning the development of certain of the organs will be discussed.

A word is necessary with regard to the identification of *Jaera marina*. In my preliminary notice ('92) I referred to it as *Jaera albifrons*, on the authority of Harger ('78). In a more recent paper by Sye ('87) it is stated that the form described by Fabricius in his Fauna Groenlandica as *Oniscus marinus* is identical with that later described by Montagu as *Oniscus albifrons*. In 1815 Leach united these two forms in the genus *Jaera*, and adopted for the species the name of *J. albifrons*, which is the authority for the name employed by Harger. If, however, the two species are identical, then Fabricius's specific name has the priority, and the entire name should be properly that employed by Möbius in 1873, *Jaera marina*. As regards

the identification of the *Porcellio* and *Armadillidium* studied, I can only say that they are the common forms of these genera occurring in New England, and lacking the literature necessary for a definite identification, I cannot state positively that they are the species named above.

The methods employed were comparatively simple. After experimenting with a number of fixing reagents, such as corrosive sublimate, hot water, picro-sulphuric acid, and others, I finally adopted an alcoholic picro-sulphuric acid, which gave admirable results, producing no distortion of the ova, and preserving the cells in an almost perfect manner. Picric acid is dissolved in 70% alcohol until saturation, and two volumes of sulphuric acid are added to every hundred volumes of this solution. The reagent is, indeed, simply Kleinenberg's strong solution made with 70% alcohol instead of with water. For staining the early stages I employed Kleinenberg's haematoxylin, generally deeply overstaining, and then carefully washing out in 70% alcohol, acidulated with hydrochloric acid. The stain is washed out more rapidly from the yolk than from the protoplasm and nucleus, which thus became very distinct. In the early stages the eggs thus treated could be cleared in oil of cloves and thus studied, but I found that in later stages, after the cells had reached the surface of the yolk, it was preferable to study them as opaque objects, using direct illumination. When the blastoderm was well formed it was possible, in the larger ova, such as those of *Porcellio*, *Armadillidium*, *Ligia*, and *Cymothoa*, to remove it from the yolk and study it as a transparent object after clearing, but in the minute eggs of *Jaera*, and to a certain extent in *Asellus*, this method of procedure was not feasible. For sectioning the ova of *Jaera*, and the later stages of the other forms, the usual paraffin method was employed. In the early stages, before the formation of the blastoderm, the brittleness which the yolk assumed under this method prevented its application to the larger eggs, and for these, after the removal of the egg-membranes, the celloidin method was employed.

Part I. — The Phenomena of Impregnation and the Formation of the Egg-membranes in Jaera.

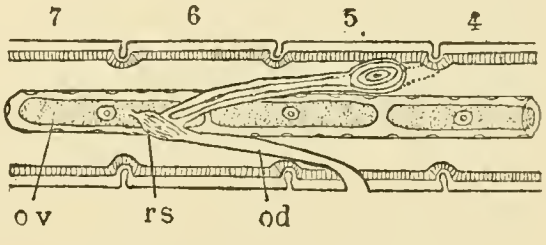
1. *The process of Impregnation in Jaera.*

On examining a series of transverse sections through an adult female *Jaera* my attention was arrested by a peculiar chitinous body, circular in section and composed of a series of concentric layers (Pl. V, Fig. 1, *Sp.*), which occurred on each side of the mid-line, just below the tergum of the fifth thoracic segment, — a short canal, at the bottom of which it lay, running forwards and upwards to open between the fourth and fifth segments. On sectioning other individuals similar structures were found, varying, however, considerably in position, sometimes having a more lateral and sometimes a more median position, sometimes near the dorsum of the body, or at other times seated more deeply and more posteriorly, but always surrounded by a cellular investment. The impression which one obtains from the examination of the relations of these structures in a number of specimens is that they are bodies which are attached to the dorsum of the female in the region indicated above, and which gradually migrate downwards and backwards into the body. In other words, they appear to be intrusive bodies, and do not, strictly speaking, belong to the category of female organs.

Sye ('87) mentions the occurrence of these structures, but has not followed their relations sufficiently to arrive at any conclusion as to their significance. He states, however, that they do not occur in the male, and were found in the female only during the breeding season, not occurring either before or after that period.

The investigation of this structure does not strictly fall within the scope of the present study, but incidentally some facts regarding it have been discovered which throw some light upon its significance, and may be recorded here in the hope that the attention of other observers will be directed towards a fuller investigation of the subject.

I believe that the chitinous capsule is a portion of a spermatophore which is deposited upon or inserted into the dorsal surface of the female, passing downwards and backwards to reach the oviduct just where it joins the ovary. I have not investigated the structure of the spermatophore in detail, nor have I observed the manner of its formation by the male, but believe that the evidence I have gathered warrants the interpretation here given. In one set of sections the capsule lay just beneath the hypodermis in the fifth thoracic segment, and extending backwards from it was a tube formed of a single layer of cells, and containing a granular mass which did not take a carmine stain. This I take to be the body of the spermatophore undergoing degeneration, the spermatozoa which it originally contained being found in the oviduct and especially in a dilated portion of it near its proximal end, which may be termed the receptaculum seminis. Fertilization takes place apparently in the oviduct; at all events I have not found spermatozoa in the ovary, though it is possible that they may pass into it during the moult which precedes oviposition, as they are stated to do in *Porcellio* and other land-forms by Friedrich ('83). The relationships of the various parts concerned are represented in the diagram above.



Relationships of the Various Parts.

The varying position of the chitinous capsules is to be explained as stages in their expulsion from the body after the spermatophores have discharged their contents. They appear to migrate down the canals originally occupied by the bodies of the spermatophores, these latter seeming to degenerate, and later they reach the oviducts, passing to the exterior finally by these. At least I have found them in sections close to the proximal ends of the oviducts, and also lying in the oviducts just internal to their external opening. I could not find any trace of the bodies of the spermatophores when the capsules had reached the oviducts, and, furthermore, the canals extend-

ing from the dorsum to the oviducts along which they passed had also disappeared in this stage, a fact which suggests the origin of the canals as an in-pushing of the hypodermis in front of the spermatophore.

If this interpretation of the observed phenomena be correct, we have an example among the Crustacea of a phenomenon which resembles not a little that termed by Whitman ('91) hypodermic impregnation, although the conveyance of the sperm to the oviduct is somewhat more direct than in the typical cases he records. Examples of the phenomenon have been found in various groups of Invertebrata, as will be seen from Whitman's paper, but, so far as I know, nothing similar to what occurs in *Jaera* has been recorded for other Crustacea. Spermatophores occur in many forms, such as the Copepods, Thysanopodous Schizopods, and some Decapods, and furthermore, copulation, during which the male rests on the dorsum of the female, is very frequent, but I know of no previously recorded case where the spermatophores are inserted upon the dorsal surface of the body.

2. *The Formation of the Egg-Membranes in Jaera.*

The newly-extruded egg of *Jaera* is of a bright grass-green color, similar to that which Rathke ('37) has described and figured for *Bopyrus* and *Janira*, and is somewhat irregular in shape, later, however, becoming oval and measuring on the average about 0.2 mm. in length by 0.19 mm. in breadth. In the center of the egg is the nucleus enclosed within a stellate mass of protoplasm, a thin peripheral layer of this same material enclosing the yolk spherules, which are mainly albuminous with a few oil globules. Sections of about half-grown ovarian eggs (Pl. V, Fig. 2) show that a delicate network of protoplasmic fibrils (*pn*) extends from the central (*cp*) to the peripheral protoplasm, the yolk granules lying in the meshes of the network in the manner described by P. Mayer ('77) for *Eupagurus;* in later stages and in the mature egg the network cannot readily be made out, being obscured by the great development of the yolk, but the peripheral protoplasm (Fig. 3, *pp*) can readily be seen during the early stages of segmentation.

The number of ova contained in the brood-pouch varies considerably in different individuals; the smallest number found was three and the greatest twenty-two, the average being about eleven. A moult apparently precedes the oviposition, as has been observed for a number of other species, but I have not endeavored to follow in detail the phenomena accompanying the extrusion of the ova. An attempt to discover the conditions which governed this process was without result; apparently it is not simply a question of time of day, *i.e.*, of light and temperature, nor can the stimulus be supplied by impregnation, since in a number of females isolated at the same time, from which later developing ova were obtained, and which must therefore have been impregnated prior to the isolation, the time of extrusion of the ova varied too much to allow of its reference to this cause.

Attention may be called to the fact that the period of egg-laying of the *Jaera marina* of our coast differs materially from that of the European form. According to Sye ('87) the extrusion of the eggs occurs in the latter in March and April, while at Woods Holl I found that it took place throughout the entire summer. At least it was going on at the middle of June when my first observations were made, and continued without interruption until the end of the first week of September, the time of my departure from the laboratory.

Eggs taken from the brood-pouch before the polar globules have formed possess but a single membrane, the chorion, which shows a decidedly wrinkled appearance, and is separated from the ovum by a considerable space. The chorion is somewhat sticky at this stage, the eggs adhering to each other and to the bottom of the watch-glasses in which they are being examined, but later this property is entirely lost. As to the origin of the membrane, my results are not very definite, since I have not been able to satisfy myself as to whether a delicate membrane was not present in ovarian eggs. In one set of sections through ovaries containing almost ripe ova I thought a very delicate membrane could in some places be discerned, and though the uncertainty was too great to warrant a definite statement, I am inclined to believe that the

chorion is a product of the follicle-cells, and is formed within the ovary.

The ova of *Asellus*, *Porcellio*, and *Armadillidium* agree perfectly with those of *Jaera* as regards the presence of membranes, the chorion being the only envelope before the extrusion of the polar globules.

Soon after the ova of *Jaera* reach the brood-pouch, the polar globules are given off, fertilization apparently taking place either in the ovary immediately before the ova leave it or else in the oviduct. How the spermatozoa pass through the chorion I could not discover, there being apparently no micropyle. Two polar globules are extruded (Fig. 4, *pg*), and one or both may subsequently divide, since not unfrequently three globules were visible, two being smaller than the third, and in some cases I observed four.

During the period occupied by the maturation of the ovum as indicated by the formation of the polar globules a second membrane, the vitelline membrane (Fig. 4, *ym*) is formed, and its relations to the polar globules possess considerable interest. In the majority of cases in which the relations were observed both polar globules were between the chorion and the vitelline membrane (Fig. 4), but not infrequently both were within the latter membrane; and in some cases one was between the two membranes and the other between the vitelline membrane and the ovum.

It seems to be a very general rule that the ova of Crustacea when extruded are enclosed within a single membrane, the chorion, which is almost certainly formed as a secretion by the follicular cells, though certain authors have referred its formation to the walls of the oviduct. A few cases occur in the literature to which I have access which form an exception to this rule. Grobben ('79) states that the egg of *Moina* possesses no membrane when extruded, but that some time after it reaches the brood cavity one is formed, which, since it must, under the conditions, be a product of the egg protoplasm, is to be regarded as the vitelline membrane. The same author ('81) has described a similar absence of a chorion in the ova of *Cetochilus*, and Della Valle ('89) finds the same conditions in

those of *Gammarus pulex*. In both these cases, as in *Moina*, a membrane forms later which must be identified as the vitelline membrane.

In regard to this latter membrane the data as to its occurrence are not quite so thorough as could be desired, partly owing to a membrane formed at a much later period, the blastoderm membrane, having been confounded with it in some cases. It seems necessary to distinguish between the two structures, the vitelline membrane being, in the strict use of the term, a membrane which is formed by the protoplasm of the egg about the time of the formation of the polar globules, and connected, as will be shown later on, with the process of fertilization. It is not my intention to enumerate the various instances in which the occurrence of this membrane has been described, but a few cases may be referred to. In *Asellus* van Beneden ('69) observed the membrane only when the process of segmentation had advanced to the eight-celled stage, but I have seen it in the egg of *A. communis* immediately after the formation of the polar globules. In *Oniscus* Bobretzky ('74) found two membranes in the youngest ova which he obtained, these membranes being apparently the chorion and the vitelline, but on the other hand Bullar ('78) finds but a single membrane in the newly extruded ova of *Cymothoa*, a second one appearing only when the embryo is fairly formed, there being apparently no true vitelline membrane, though it is possible that it may have been overlooked, since Bullar did not have an opportunity of examining ova in the early stages of segmentation.

In the Decapod Crustacea there are more definite statements as to the absence of this membrane. Ishikawa ('85) found two membranes in *Atyephira*, and Lebedinski ('90) has described the same number as occurring in *Eriphya*, and it may be presumed that in both these cases we have to do with a chorion and a vitelline membrane. In the Lobster, however, Bumpus ('91) found no trace of a vitelline membrane, and the same result followed the researches of Kingsley ('87) on *Crangon*, Cano ('93) on *Maja*, and apparently those of Mayer ('77) on *Eupagurus* and Herrick ('92) on *Alpheus*, to mention only some of the more recent observations.

What all the conditions may be which determine the formation of the vitelline membrane is at present obscure, but the *primary* condition is understood more especially through the observations of Fol and the Hertwigs ('87) to be normally a stimulus imparted to the egg protoplasm by the spermatozoön. Herbst ('93) has shown that it is possible to imitate this natural stimulus and call forth the membrane in unfertilized eggs by subjecting them to the action of various chemical substances such as Benzol, Toluol, Xylol, and others; and the Hertwigs found that exposure to the action of certain poisons such as cocain and chinium sulphate deprived the protoplasm to a greater or less extent of the power of responding to the normal stimulus. The membrane appears to be the outer layer of the protoplasm of the ovum, hardened or thrown off as a result of a stimulation of the protoplasm. Such a cause for its formation explains its relations to the polar globules of *Jacra*. It is entirely unrelated to their formation, as their varying position within or without the vitelline membrane demonstrates, though it is possible that their formation, that is to say, nuclear division, may also be more or less influenced by the penetration of the spermatozoön. Why the membrane does not form in *Homarus* and other forms it is difficult to say; it can hardly be a case of inhibition of the stimulus due to the pressure of a considerable amount of yolk, since the ova of *Crangon* have a less abundant yolk than those of *Porcellio*, yet the former do not develop a vitelline membrane while the latter do. Still it is possible that the yolk may have some effect, since it seemed that in *Porcellio* the formation of the membrane was in some cases very much belated, not appearing until after segmentation had begun; opportunities for making conclusive observations on *Porcellio* were, however, not afforded me.

Part II. — The Segmentation and Formation of the Germ-Layers in the Isopods.

1. *The Segmentation and Formation of the Germ-Layers in Jaera.*

Shortly after the extrusion of the second polar globule the first segmentation occurs, its plane passing in the usual manner through the point at which the polar globules were extruded, and lying, therefore, at right angles to the longer axis of the egg. The division affects, however, only the nucleus and the protoplasm which immediately surrounds it, there being no indication of the division upon the surface of the ovum, a state of affairs which persists through several divisions. For the sake of convenience in description, however, the two nuclei with their surrounding protoplasm (Pl. V, Fig. 5, *A*, *C*) will be spoken of as cells, it being understood that the ovum at this stage and throughout several subsequent stages is in reality a syncytium.

The second division results in the formation of four cells and is likewise confined to the nuclei and the protoplasm in their immediate vicinity. The axes of both spindles are evidently at right angles to the direction which the spindle of the first division held, but after the division is completed the lines which may be imagined as joining the four cells into pairs are not in any case observed by me parallel to each other. Thus, in some cases when two of the cells, derived from the same parent cell, were in clear focus, the other two were also visible, one being in almost equally clear focus while the other was somewhat obscure, and the lines joining the members of each pair were evidently inclined to one another at an angle which varied considerably in different cases. In other eggs, however, when two of the cells were in clear focus only one other could be perceived, the fourth being directly below it and only brought into view by rolling the egg through an angle of about 90°; in other words in these cases the lines joining the cells in pairs are exactly at right angles with one another. These two conditions are shown in Figs. 6 and 7 of Pl. V, the two

cells *A* and *B* being the products of the original cell *A*, and *C* and *D* the result of the division of the original *C* of Fig. 5. It would be interesting to know definitely whether these two arrangements represent two modes of segmentation; or whether the cases in which the inclination of the axes of the two pairs to each other is comparatively slight, are simply stages in rotation through an angle of 45° of each of the two pairs of cells formed by a typical meridional division. I did not succeed in obtaining ova in the spindle stage preceding this division, and therefore cannot answer this question positively, but from the circumstantial evidence at my disposal I am inclined to believe that there is a rotation, but also that in a number of ova it remains incomplete.

In the cases in which the rotation has reached its fullest extent some interesting features are brought to light when a comparison is made with the corresponding stages of other ova. The existence of a cross-furrow has recently attracted considerable attention in connection with its significance as indicating a "spiral" form of cleavage. The rotation of the egg of *Jacra* may be regarded as an extreme form of "spiral" cleavage, and it becomes of interest to note the relationships of the furrows which separate the various cells. In this particular egg no cross-furrows exist in reality on account of the nature of the segmentation, but it is not difficult to imagine what their relations would be were the cleavage holoblastic. Practically the same condition would obtain which has been described by Ludwig ('82) for the ovum of *Asterina* in the four-celled stage, and this condition may be regarded as due to the rotation of each pair of cells through an angle of 45°, the arrangement thus being essentially similar to that described by Wilson ('92, p. 452) for *Nereis*. In the figures of *Jacra* the conditions appear to be slightly different, but this is due to the slightly different position in which the egg is drawn, a position which may be imitated by rotating the *Nereis* egg through 45°, when *A* and *B* will lie in one plane and *C*, *D*, in another plane at right angles to the former.

In *Jacra*, then, we have to deal with a rotation of each of the two pairs of cells through an angle of 45°, and the arrangement

is strictly comparable to that of *Nereis*. That this is so is evidenced by the relation of the antero-posterior axis of the future embryo to the first segmentation plane, which is the same as in *Nereis*, a fact which can, however, be more readily perceived when the next segmentation has been completed.

In Fig. 8 is shown a preparation in which the division into eight cells is not quite completed, the cells being still united in pairs by a short band of protoplasm. It will be seen that *A* and *B* have divided in such a way that their spindles must have been parallel, one of the daughter-cells of each lying immediately below the other, and being thus concealed from view. This division is practically an equatorial one. The spindle of cell *C*, however, assumed a position at right angles to those of *A* and *B*, and thus underwent what may be considered a third meridional division, while the spindle of *D* was inclined at an angle of 45° to the other three. As the result we get the arrangement which is represented in Fig. 9, taken from an egg which was rotated slightly so as to bring all four cells resulting from the division of *A* and *B* into view. It will be seen from this that the cells have arranged themselves around the long axis of the egg, an axis which represents the longitudinal axis of the future embryo, and which stands at right angles to the axis indicated by the polar globules. Around one of the new poles of the egg, which the later development shows to correspond to the anterior extremity of the embryo, four cells, *A*, *a'*, *B*, *b'*, are arranged at almost equal distances, and near the posterior pole is a second circle of three cells, *C*, *c'*, and *d'*, also almost equally spaced, while the eighth cell, *D*, occupies an almost polar position, lying, however, a little to one side of the actual pole. This cell is destined to give rise to the vitellophags, by which term its descendants may hereafter be denoted.

We have seen that the first division-plane was parallel to the shorter axis of the egg, at the extremity of which were the polar globules, while the second plane was parallel to the larger axis, and we now see that the longitudinal axis of the future embryo corresponds with that of the second division-plane, an arrangement which agrees with what occurs in *Nereis*, *Crepidula*,

and *Umbrella*. It has been shown, however, by the observations of Miss Clapp ('91) that too much importance has been attached to the question of the axial relations of the ovum and the embryo, and further consideration of this point may be omitted. But not only is the future longitudinal axis marked out in the eight-celled stage, but the dorso-ventral axis is also clearly indicated by the position of the vitellophag cell, that side of the egg upon which it lies being the future ventral surface of the embryo. Unfortunately I have not been able to ascertain what relation this surface bears to the point of extrusion of the polar globules, since these structures cannot be discovered in the preserved ova upon which I worked; it would be interesting to know if in this particular, also, *Jaera* resembles the forms mentioned above.

The relations of the cells to the yolk and the peripheral protoplasm in the eight-celled stage are shown in the section represented in Fig. 10. The peripheral protoplasm (*pp*) is still to be seen as a distinct layer covering the surface of the yolk mass, and at a short distance below it, imbedded in the yolk, are masses of protoplasm surrounding the nuclei. During the segmentation these structures have passed peripherad, and in the eight-celled stage the protoplasmic masses surrounding them have almost reached the surface, their connection with the peripheral protoplasm by means of slender processes being very distinct. Other processes radiate into the yolk, and though actual anastomoses of the processes from different masses were not observed, the presumption is very strong that they exist. The central portion of the yolk presents a very different appearance from that which encloses the cells, having broken up into small masses, from which apparently all protoplasmic matter has been withdrawn, there being in *Jaera*, as in many other Crustacea (*e.g.*, *Moina*), a central mass of yolk entirely destitute of protoplasm.

In the next stage a division of all the cells again takes place, and a sixteen-celled stage is the result. The greatest interest attaches to the cells situated at the posterior pole of the egg, and to these alone our attention need be directed. In a number of eggs in which the segmentation had just been completed,

or in which traces of the spindle were still visible, I found at the posterior pole the arrangement which is represented in Fig. 11. The two cells *vi* are the products of the division of *D* of the preceding stage, and represent the vitellophags ; they are surrounded by a circle of seven cells, six of which result from the division of *C*, *c'*, and *d'* of the eight-celled stage, while the seventh is derived from one of the cells of the anterior pole, probably *A*. In a number of eggs, however, I found the number of cells forming the ring to be six only (Fig. 12), a number which agrees with what is found in the next stage. It seems that shortly after the completion of the division there is a migration or rearrangement of certain cells, one of those which at first formed the ring leaving it and migrating towards the anterior pole of the egg. My reason for supposing this to be the case is that I have found the seven-celled ring in ova in which the division was not quite completed, while the six-celled ring was only observed in fully divided eggs, and furthermore it will be seen that the next stage of development can be derived directly only from a six-celled ring. The cell which leaves the ring is not that (*A'*) which came into it during the division from the anterior-pole, but so far as I can discover it is one of the cells produced by the division of *c* (*i.e.*, c^2 of Fig. 11). From Fig. 12 it might be supposed that it was *C* of Fig. 11 that had migrated out of the ring, but this appearance is, I believe, due to an alteration of the position of *C* after c^2 has moved away. From the observation of a number of eggs, and noting the position of the cell which persists in the ring with relation to the two endoderm cells, I have come to the conclusion that c^2 is the one which disappears.

What the significance of this migration may be I cannot even suggest. Why a cell belonging to the anterior hemisphere should enter the posterior one, and *vice versâ*, I cannot understand. The result of the process is, however, a differentiation of the germ-layers at this early stage. At or in the near vicinity of the posterior pole are the two vitellophag cells ; the six cells surrounding them will give rise to the mesoderm and the liver endoderm ; while the eight remaining cells occupying the anterior hemisphere of the ovum will form the ectoderm.

In the 32-celled stage a remarkable difference of appearance supervenes, depending upon the cells having at length reached the surface of the yolk and fused with the peripheral layer (see Fig. 20), thus allowing a superficial indication of the segmentation to appear. The eight ectoderm cells of the 16-celled stage have given rise to sixteen cells (Fig. 13, *Ec*), which have a characteristic appearance. Each has a more or less perfect hexagonal outline in surface view, and in the center of the area enclosed by cell-boundaries is the mass of protoplasm surrounding the nucleus, which I have spoken of hitherto as the cell. This mass of protoplasm is not, however, sufficiently large to cover the entire surface of the cell, but sends off numerous radiating processes which come into contact with those of other cells, so that, even at this stage, when the true cell-boundaries are distinguishable, the ovum is a syncytium, a fact of which sections give ample evidence. The six dark mes-endoderm cells of the 16-celled stage divide in such a manner as to form a circle of twelve cells (Fig. 13, *MEn*), placed somewhat obliquely to the antero-posterior axis, while the posterior pole is occupied by four vitellophag cells (*vi*), which are not, however, arranged exactly around the posterior pole, but incline slightly towards the ventral surface. A marked difference in capacity for staining also becomes evident in the cells composing the egg at this stage. The twelve mes-endoderm cells, as the cells marked *MEn* in the figures may be termed, are distinguished by the very deep tint which their protoplasm shows, as well as by the apparent absence of processes radiating from it, while the vitellophag cells show, on surface views, hardly any protoplasm at all, the darkly-stained nuclei standing out prominently upon an almost unstained ground. The ectoderm cells are intermediate between these two extremes, their protoplasm being distinctly visible, but assuming a much fainter stain than the mes-endoderm. This peculiarity is in part due to the different concentration of the protoplasm around the nuclei in the three kinds of cells. As sections (Fig. 20) show, the nuclei of the mes-endoderm cells (*MEn*) are surrounded by a large quantity of protoplasm, from which slender processes pass outwards to unite with a thin layer of protoplasm which

forms the cell-boundary and from which other delicate processes extend into the yolk, probably uniting with processes of other cells. The ectoderm cells (*Ec*) have much less protoplasm around their nuclei, and the protoplasmic processes are, as a rule, longer and stouter than those of the mes-endoderm while a layer of protoplasm marking the cell-boundaries is not so distinct, though indications of it may be seen. The vitellophag cells, on the other hand (*vi*), seem to consist simply of nuclei imbedded in the peripheral protoplasm, and in sections I could distinguish no processes in connection with them. The segmentation, though appearing from the surface to be total, is in reality not at all so, the greater portion of the yolk taking no part in it, and indeed being apparently destitute of protoplasm.

In the next stage (Fig. 14) the vitellophag cells (*vi*) have increased to eight, the mes-endoderm (*MEn*) is composed of two circles, each consisting of twelve cells, and the number of ectoderm cells has also been doubled, so that a 64-celled stage is reached. This stage does not call for any more detailed description, except that it may be pointed out that the various cells still retain the staining peculiarities which distinguished them in the preceding stage.

The 64-celled stage is the last one in which the division involves every cell, that is to say, in which the increase of cells follows a geometrical progression. In the next stage the vitellophag cells do not take part in the division, and, in fact, do not divide again for some time. The next stage, consequently, is composed of 120 cells, a view of the posterior pole of an egg passing into it being given in Fig. 15. It will be there seen that the cells of the two mes-endoderm circles are dividing in such a manner that each circle will eventually be formed of twenty-four cells. It will be observed, however, that one of the cells of the posterior circle is dividing in a somewhat different plane from all the others, so that one of the daughter cells (*l.en*) will encroach upon the area occupied by the vitellophags. The significance of this cell is a little doubtful, since I have not been able to follow the fate of its products. I believe, however, that it is destined to give rise to the endoderm which will form the so-called liver lobes, and

consequently have indicated it in the figure as the liver endoderm (*l.cn*). My reason for this belief is derived from the analogy which may be traced between this cell and the cells which give rise to the liver in *Astacus*, in which form the origin of the liver has been clearly traced by Reichenbach ('86). After the invaginated cells have begun to assume their vitellophag function, there is to be seen in the ventral wall of the invaginated sack a layer of columnar cells which do not ingest the yolk-granules, and which form what Reichenbach terms the entoderm plate. From these cells the liver lobes arise. If, as I think must be done, the vitellophag cells of *Jaera* be compared to the invaginated cells of *Astacus*, which form the secondary yolk-pyramids, then the relations of the liver endoderm of *Jaera* correspond very closely with those of the endoderm plate of *Astacus*, a point which may be more clearly seen in the succeeding stages (*e.g.*, Fig. 17). In *Astacus* the vitellophag cells and the cells of the entoderm plate form a continuous layer, but in *Jaera* the vitellophags scatter irregularly through the yolk, and the continuity of the two parts is broken. Making due allowance for this difference, I think there is sufficient similarity between the two structures, the liver endoderm of *Jaera* and the entoderm plate of *Astacus*, to suggest their identity. It is anticipating somewhat to enter upon a discussion of the origin of the liver lobes here, but it may be stated that the cells from which they arise *in situ* in *Jaera* and other Isopods cannot readily be distinguished from the mesoderm cells which lie in their immediate vicinity. As will be seen later, there is a migration forward of the majority of the mesoderm cells to form the mesodermal tissues of the anterior or naupliar portion of the body, and in this migration the cells of the liver endoderm, it may be imagined, share, taking up their position on each side of the body and proceeding to ingest yolk. In their origin they are closely related to the mesoderm cells and resemble them in appearance, whence the difficulty of distinguishing them, when they have reached their definite position, from the adjacent mesoderm cells.

In my preliminary notice ('92) of some of the observations recorded in this paper, I described the row of dark cells in the

32-celled stage as mesoderm, while the vitellophags I regarded as representing the entire endoderm. I now believe that this view was incorrect, one of the so-called mesoderm cells containing endodermal material as well as mesodermal. This cell is evidently situated near the ventral mid-line, the remaining cells of the circle being purely mesodermal. In the stage at present under discussion the endoderm (*l.en*) and the vitellophags (*vi*) become finally separated from the mesoderm (*me*) by the oblique separation off of the liver endoderm cell, and the differentiation of the germ layers is thoroughly established. Fig. 16 is a side view of an ovum of this stage in which the division has been completed, and it is seen that the staining properties of the cells remain very nearly the same as in earlier stages, though some of the ectodermal cells in the mid-ventral line just anterior to the mesodermal circles are no longer readily to be distinguished by their staining powers alone from the mesoderm cells.

Beyond this stage I have not been able to follow the divisions stage by stage, and the next stage figured is somewhat more advanced than the last, and probably represents the results of two cleavages. Interesting changes which will result in the differentiation of the embryo are now beginning. In Figs. 17 and 18 are represented two views, a ventral and a dorsal, of the same egg. In the ventral view one sees a noticeable increase of the area occupied by the mesoderm (*me*), or at least by darkly-staining cells which appear to be mesodermal, though it is possible that a few of them may be ectodermal cells which have stained more deeply than usual. For the most part, however, these cells are mesodermal, and in the mid-line there projects backwards from them the liver endoderm (*l.en*), which is now composed of several cells. The number of vitellophag cells still remains at eight, and in front of the mesoderm are seen the ectoderm cells, which show the anastomosing processes so well-marked in the 32-celled stage (Fig. 13). In comparison with the stage represented in Fig. 16 the presence of these processes is very marked, but it seems probable that that figure represents an ovum preparing to divide, and that in the resting stage the syncytial condition

of the ectoderm becomes as well-marked as in Fig. 17. The dorsal view (Fig. 18) presents a very different appearance from the ventral. In the first place, the ectoderm cells are much more widely separated from one another, and secondly, the mesoderm no longer forms a complete ring, but a break in its continuity has appeared in the mid-dorsal line, and, furthermore, it will be seen that the band thus formed tapers rather suddenly toward either extremity. These appearances can only be explained satisfactorily by supposing that a migration of the ectodermal and mesodermal cells is taking place towards the ventral surface, or to express it slightly differently, that all the mesoderm cells and part of the ectoderm is concentrating upon the ventral surface to form the embryo.

This concentration has become more marked in the stage represented in Fig. 19. The preparation is figured from the ventral surface, and it is seen that the mesoderm band has become broader along the ventral mid-line, or, at any rate, that it is composed of a greater number of cells in that region than it was in the preceding stage, and at the same time the bands have thinned out considerably at the sides where before they were broadest. The liver endoderm (*l. en*) is still visible, though less prominent than before, and the vitellophags have divided, as may be seen from their marked approximation in pairs. The ectodermal cells on the ventral surface have now come into close apposition so as to form a plate lying in front of the mesoderm band, the front edge of the plate being somewhat deeply notched in the mid-line. The cells composing it are well defined, and in the preparations are distinctly separated from each other, possessing no protoplasmic processes as in preceding stages, and sections show that they now give off no processes into the yolk, which seems to be entirely destitute of protoplasm and forms a central purely nutritive mass, upon the surface of which the cells lie. So far as the ectoderm of the plate and the mesoderm are concerned the syncytial condition no longer exists, though apparently the dorsal ectodermal and the vitellophag cells still are united through the intervention of the peripheral protoplasm. As regards the ectodermal cells, they are rather widely scattered, and their protoplasm is

hardly visible in surface view, though more distinct than that of the vitellophags.

Sections through this stage, or through one very slightly later (Pl. VI, Fig. 21), show that during the concentration of the mesoderm towards the ventral surface it becomes several layers thick, forming a thickening of cells projecting into the yolk, all the cells, however, being well separated from this latter material. In front of this mesodermal "plug" lie the cells of the ectodermal plate (*cc*) arranged in a single layer, and it is further to be noted that the yolk at this stage contains no cells whatever, nor are any to be found in it by a most careful study of serial sections through ova in what may be termed the blastula stages (Pl. V, Figs. 13–16). All the cells produced by segmentation reach the surface in *Jaera*, and in this respect my observations are in harmony with those of the more recent students of Decapod embryology, such as Weldon ('92) and Herrick ('92), and in opposition to those of Kingsley ('87). In one batch of eggs taken from a single individual, and which were in about the same stage of development as those represented in Figs. 17 and 18, I did find in several cases a single cell almost in the center of the yolk below the mesoderm band. The arrangement of the superficial cells, however, differed distinctly from those of other eggs in the same stage of development obtained from several individuals, and I believe these ova to have been abnormal. Whether or not they would have proceeded on to complete development I was, of course, on account of the method employed, unable to determine.

In a preliminary notice of this paper published some time ago (McMurrich, '92) I ascribed the formation of the mesoderm plug to the division of the mesoderm cells parallel to the surface of the ovum, *i.e.*, to delamination. Further observation has, however, led me to doubt the perfect accuracy of this statement. Division of the mesodermal cells forming the plug occurs, it is true, but it seems probable that during the concentration of the mesoderm towards the ventral surface some of the cells are, so to speak, elbowed out of their original superficial position and forced below the surface, and to this process rather than to a tangential division I ascribe the formation of the plug.

When the concentration is complete the mesoderm forms a somewhat oval mass of cells whose anterior edge is surrounded by a row of ectodermal cells which are the most posterior cells of the ectodermal plate. In *Jaera* on surface view these cells are not specially differentiated from the other ectodermal cells of the plate, but the further development shows that they possess a somewhat special function, and that they correspond to the ectodermal teloblasts which become evident later on, and which have been described in *Cymothoa* by Patten ('90). They now begin to divide in a direction at right angles to the long axis of the embryo, and by repeating this division give rise to rows of cells extending forward from them. By this process the teloblasts are themselves forced backward over the mesendodermal plug, which thus becomes covered by ectoderm. About the time, however, that the mesendodermal plug is about half covered, the vitellophag cells, which, up to this time, have retained their original position, begin to migrate into the interior of the yolk, as is shown in the section represented in Pl. VI, Fig. 22. In this section one sees anteriorly a row of ectoderm cells (*ec*) which represent the ectodermal plate, and behind them two cells (*tr*) which form one of the rows of cells produced by the division of the teloblast (*T*). This cell appears considerably larger than the other ectodermal cells, but so great a difference as occurs in this particular case is not as a rule noticeable. Below the teloblast and behind it are found a number of mesoderm cells (*MEn*) representing the mesendodermal plug, but which are much more loosely aggregated than they were in the section represented in Fig. 21. The chief interest of the section, however, lies in the arrangement it shows for the vitellophag cells (*vi*). Two of these are seen; one still occupies a position at the surface, while the other has sunk into the yolk a short distance. The example of the latter is followed later by the other vitellophags, and it may be again remarked that when they begin their immigration there is no trace of cells in the interior of the yolk, nor have I succeeded in finding evidence that any of the vitellophags come from other regions of the blastoderm. In later stages the vitellophags are certainly more numerous than they were

at the time of immigration, but I believe that this is entirely due to a subsequent multiplication, and that all the vitellophags trace back their origin to the cell *D* of the eight-celled stage.

By the time the ectoderm has grown back over the mes-endoderm plug the embryo has the appearance represented in Fig. 22, in which the area occupied by the mesoderm is indicated by the dark shading. The ectoderm cells covering this region are manifestly arranged in rows as they are also towards the sides, while anteriorly they become more scattered, though this is not well shown in the figure. The penultimate row of cells seems to be the teloblast row, though I could not make out a perfect correspondence between them and the rows of cells extending forward from them. It will be noticed, however, that the multiplication of those nearer the middle line has taken place somewhat more rapidly or to a greater extent than that of those situated towards the sides, and the posterior edge of the ectoderm plate has now become convex backwards instead of concave as it was originally. In front of the mesoderm and to the sides a series of rows of cells passing forwards and outwards can be seen, and at the extremity of these is a patch of larger cells which will later give rise to the eyes and which are clearly seen in the side view (Fig. 23, *E*). These rows of cells leading towards the eyes were not always, however, as clearly marked as in the embryo from which this drawing was made. One other fact in connection with the preparation requires mention, and that is that the cells of the mesendodermal plug are much less closely aggregated than in earlier stages and seem to have a tendency to scatter themselves, so that the shaded area of the figure has a greater extent than that originally occupied by the plug.

The division of the teloblasts progresses in later stages, and a layer of ectoderm, whose cells are arranged in regular longitudinal and transverse rows, extends backwards over the yolk. In Figs. 24 and 25 is represented an embryo in which the blastoderm has extended about two-thirds of the way around the yolk, Fig. 24 representing the anterior half of the embryo, and Fig. 25 the posterior half. Looking at the anterior half

one sees that the cells of the anterior portion of the body are arranged very irregularly, but form three bands which enclose a triangular area whose apex is directed backwards and whose cells are scattered about irregularly. The two bands which form the sides of the triangle are the ventral lateral bands which have been so often figured in the early stages of the Decapods and in which the naupliar appendages and nervous system will form, while the transverse band forming the base of the triangle is apparently not so well marked in the Decapods, though evidently distinct in *Alpheus* according to Herrick's ('92) figures and descriptions. At the basal angles of the triangle are the "Anlagen" of the eyes (*E*). This anterior region represents then the naupliar region of the embryo, and behind it comes what has been termed by Bergh ('92) the metanaupliar region, whose ectoderm is markedly arranged in rows and has resulted by the growth of the teloblasts. Examining the anterior portion of this region, one finds that there are about eleven rows of cells which extend farther forwards than the others. In the embryo figured there seemed to be only ten of these longer rows, but this seems to have been an abnormality if it can be so called, eleven being probably the typical number. Further back other rows are to be found on each side, those nearer the middle line being longer than those further laterad. At the end of each row is a teloblast (Fig. 25, *T*) slightly larger than the other cells of the row and frequently showing karyokinetic phenomena. The number of teloblasts and accordingly of the rows is liable to a certain amount of variation apparently. A central teloblast with its row is generally well marked, as may be seen in Fig. 26 (*cT*) drawn from a somewhat older embryo than Fig. 25, and it may be stated here that the cell row arising from this gives rise to a "Mittelstrang" recalling what has been described in other Invertebrates. On each side of this central teloblast there are about eleven or twelve lateral teloblasts, there being typically the same number on both sides, though not infrequently one finds, for example, eleven on one side and twelve on the other, as in Fig. 26. Behind the teloblast row is a varying number of ectoderm cells arranged somewhat irregularly. They are

somewhat more numerous in later than in earlier stages, as may be seen by comparing Figs. 26 and 25 (*Ec'*), but their exact origin I have not been able to determine; it seems possible that they may be ectodermal cells which the teloblasts have pushed before them in their growth over the surface, a view which their increasing number in older stages would justify. I cannot, however, advance any definite proof of this idea.

As regards the mesoderm the results obtained from the study of *Jaera* have been by no means satisfactory, the smallness of the egg rendering proper manipulation exceedingly difficult. It is, however, easy to determine that the mesoderm plug no longer exists in the stage represented in Figs. 24 and 25, and that mesoderm cells are to be found in this stage beneath the ectoderm of the naupliar region of the embryo. That these two facts stand in intimate relation with one another I have good reason to believe. Sections show that in stages slightly younger than Fig. 24 the cells of the mesoderm plug are becoming separated and are extending forwards into the region covered by the ectoderm plate. This process continues in later stages, and one finds eventually only a few scattered cells in the region originally occupied by the mesendodermal plug, while anteriorly the cells have become quite numerous. The greater part of the mesoderm contained in the mesoderm plug goes accordingly to form the naupliar mesoderm, and so far as could be discovered *all* the naupliar mesoderm in *Jaera* is derived from this source. The liver endoderm in stages later than Fig. 19, is not distinguishable and apparently accompanies the mesoderm in its distribution, taking up its position at the junction of the naupliar and meta-naupliar regions where later the liver Anlagen appear. Of the origin of the meta-naupliar mesoderm little could be determined. One finds the mesoderm arranged in masses on either side of the middle line in Fig. 26 (*Me*), and from what is known to occur in other forms it may be supposed that these masses are the result of a teloblastic division such as Patten ('90) has described for *Cymothoa*. I was unable, however, to make out the mesodermal teloblasts in surface views of *Jaera*, and though

it is certain, I think, from the sections I possess that they exist, yet I have not been able to get a clear idea from *Jaera* either as to their arrangement or as to their origin.

The development of *Jaera* has now been traced up to a period in which the germ layers have assumed their general distribution. Further changes are mainly of a histogenetic character and may be postponed for the present until an account has been given of the development of *Asellus*, *Porcellio*, and *Armadillidium*.

2. *The Segmentation and Formation of the Germ-layers of Asellus Communis.*

So far as I am aware, there are no published descriptions of the early development of the common *Asellus* of this country, first described by Say as *Asellus communis*, though several more or less complete accounts have been given of the European *A. aquaticus*. Rathke ('34), Dohrn ('67), and Sars ('68) did not make any definite observations upon the segmentation, but Van Beneden ('69) describes accurately its general features, showing that it is of the typical centrolecithal character and regular, though he did not attempt to follow it out in detail. The latest author who has written on the subject, Roule ('89), has certainly not advanced our knowledge of it. His statements in the brief contribution he has published are so remarkable and so little in harmony with those of Van Beneden and with what might be expected from analogy with other Crustacea, that one involuntarily distrusts them. They are to be explained probably as due to failure to study in surface views stained ova, the only method by which the phenomena of the early stages can satisfactorily be made out.

My observations on *Asellus communis* are, I regret, not quite so complete as these on *Jaera*, inasmuch as I failed to obtain a few stages; they are, however, sufficiently connected to allow of a close comparison with *Jaera*, which was the object I had in view in undertaking them. The early segmentation is step for step identical with that occurring in *Jaera*, so far as that form of segmentation followed is concerned. I have reason to believe,

however, that in this species also variations occur in the segmentation, certain cells in different eggs dividing in different directions. I have not, owing to technical difficulties, attempted to follow out these variations, but have confined my attention to that variety which resembled what has been described for *Jaera* and which seems to be the most typical.

The center of the yolk is occupied in the unsegmented egg by a mass of protoplasm containing the segmentation nucleus and prolonged at the surface into a number of branching processes, which undoubtedly are continued through the yolk to form a network in the meshes of which are situated the yolk granules. I was not able to detect this network in mature ova, but a peripheral zone of protoplasm, somewhat thinner relatively than that of *Jaera*, was readily observable and is probably, as in that form, in organic continuity with the central mass. The first cleavage divides this latter into two portions (Fig. 28, *A*, *C*), its plane being in this case apparently also at right angles to the long axis of the future embryo. The second division gives rise to four cells (Fig. 29, *A*, *B*, *C*, *D*), two of which lie in a plane at right angles to that of the other two, while the third division produces eight cells, four of which (*A*, *a'*, *B*, *b'*) are arranged in a circle near the anterior extremity of the embryonic axis, three others (*C*, *c'*, *d'*) in a second circle near the posterior extremity of this axis, while the eighth (*D*) lies slightly to one side of the extremity of the axis, the whole arrangement being identical with that of *Jaera* in the same stage (*cf.* Fig. 9). In the 16-celled stage the arrangement is slightly different from what obtains in *Jaera*. All the cells divide as in that form, and there are produced two circles, each of four cells, in the anterior half of the egg, while at the equator are two other cells, which arise from two of the cells forming the circle of three in the eight-celled stage. Behind these is a circle of four cells which correspond in position to the mesodermal circle of *Jaera*, but have not the same fate, while near the posterior pole are two cells (*D* and *X*) corresponding in position and history, but not exactly in function with the endoderm cells of *Jaera*. I have not observed any shifting of cells such as I have described for *Jaera*,

and the principal difference in the two forms seems to be the smaller number of cells in *Asellus* in the circle surrounding the products of *D*.

The next, or 32-celled stage, I have not, unfortunately, succeeded in obtaining, but from preparations of the 64-celled stage I have been able to determine what the divisions may have been. In Fig. 32 is a view of the 64-celled stage in which the cells which arise by the division of each of the cells of the 32-celled stage are bracketed. Disregarding all other cells than *D* and *X* of Fig. 31, each of these divides into two, so there are four cells which correspond cytogenetically to the four vitellophag cells of the 32-celled stage of *Jaera*. At the next division these divide into the eight cells represented in Fig. 32, as D^1 D^3 D^5 D^7 $X^{1\text{-}4}$, which present a well-marked differentiation, the cells indicated by *D* being much smaller than those which result from *X*. It will be noticed the *X* and *D* groups of cells no longer are situated at the extremity of the oval egg, but occupy almost the middle of one of its faces, that namely which is to be the ventral surface of the embryo. Whether this change of position is due to a shifting forward of all the cells over the yolk, the axes of the egg remaining as before, or whether it is only an apparent shifting due to an alteration of the shape of the yolk, I am unable to say, but incline towards the latter idea. It is also noticeable that the segmentation has now manifested itself at the surface, the surface of the yolk being divided into areas corresponding to the cells, a central mass of yolk, apparently destitute of protoplasm, remaining unsegmented. In *Jaera* the superficial segmentation made itself manifest in the 32-celled stage; whether it appears in the same stage in *A. communis* I am unable to state, but it seems not improbable that it does. In *A. aquaticus*, according to Van Beneden ('69), it appears in the 16-celled stage.

In the next stage 128 cells are formed, the *D* group having now increased to eight (Fig. 33, D^1–D^8) and still remaining distinguishable by their smaller size. The *X* group I was not able to identify in this stage, at which, indeed, a very unfortunate gap occurs in my observations. This is all the more

unfortunate in that it is just after this stage that the differentiation of the mesoderm takes place, and I am obliged to depend upon circumstantial evidence in attempting to ascertain its origin. Shortly after the 128-celled stage, the concentration of the cells towards the ventral surface, to form the blastoderm, begins. By the time this has become well marked the differentiation of the mesoderm has made itself apparent. In Fig. 34 is represented the youngest blastoderm I observed. It forms a somewhat oval disc, whose longer diameter is at right angles to that of the future embryo. In the center is a patch of cells (*vi*) which stain more deeply than the rest and which I take to correspond to the vitellophag cells of *Jaera;* in front of them lies a row of cells (*MEn*) somewhat smaller than those still farther forwards, and this row I believe to represent the mesoderm, or more probably, as in *Jaera*, they are mesendodermal. In the blastoderm figured the row seems to be composed of six cells, a number which suggests a close comparison with *Jaera*, in which the same number was found when the mesoderm was first differentiated. As to the origin of the mesendodermal cells I can only suppose that they belong to the *D* group, and that the four *D* cells of Fig. 32 contain all the mesoderm and endoderm of the future embryo. The small size of the mesendoderm cells, and the fact that they and the vitellophag cells combined are just about sufficiently numerous to account for two divisions of the *D* group, suggests that this number of divisions have intervened between Figs. 33 and 34, and judging from the appearance of Fig. 34 there can hardly have been a smaller number, though I did not attempt to enumerate the ectoderm cells. This method of determination from circumstantial evidence of this kind is, I am aware, exceedingly unreliable; but I believe in this case it allows of accurate conclusions, and that from the *X* cell of Fig. 31 ectoderm cells and from the *D* cell mes-endoderm and vitellophags result.

In a slightly later stage (Fig. 35) the vitellophags are still clearly distinguishable, and the mesodermal row (*Me*) has become much longer, now forming a curved line of about fourteen (?) cells surrounding the anterior portion of the endoderm.

In front of these another row of cells (*T*) is noticeable, consisting of exactly *eleven* cells. This is the ectodermal teloblast row, and it is always symmetrical, a central cell (*cT*) being readily distinguishable with five cells on either side of it. At this stage no further differentiation is noticeable, but a little later the arrangement represented in Fig. 36 is found. Here a central, darkly-staining mass of cells may be found, which consists of both mes-endoderm and vitellophags. It is several cells thick, and represents the mesendodermal plug *plus* the vitellophags of *Jaera*. About the center of the mass a distinct depression was visible in the specimen figured, but it does not correspond to any deep invagination, but seems to be formed by the withdrawal of several cells from the surface at this point. The mesoderm is no longer distinguishable from the endoderm either by its staining properties or by its arrangement, but it seems highly probable that the anterior part of the mass is mesendodermal, while the posterior is composed of vitellophags. What has happened is that the mesodermal row of Fig. 35 has undergone a concentration towards the middle line similar to what occurred in *Jaera*, a multiplication of the cells having taken place at the same time. A careful examination of the anterior edge of the mesendodermal mass shows a somewhat irregular row of *eight* cells (*meT*), a number which suggests the possibility of these being the eight mesodermal teloblasts which, as will be seen later, give rise to the meta-naupliar mesoderm. The ectodermal teloblasts (*T*) are even more distinct than in the last stage, and have arranged themselves somewhat differently. The five cells of each side have come closer together, and have separated from the central teloblast (*cT*), and, furthermore, each of the lateral teloblasts has budded off a small cell (*tr'*), the first of a teloblastic row. The lateral teloblasts and their progeny form two rows each consisting of five cells lying in front, and somewhat laterally to the mesoderm plug. Extending from these rows laterally can be noticed on each side a band of ectoderm cells, showing a slight tendency to be arranged in rows, but nevertheless with a good deal of irregularity. These two bands are the lateral ventral bands of the naupliar region of the embryo. The cells which are to give

rise to the eyes cannot be distinguished at this period, nor is a distinctly marked transverse band, such as occurs in *Jaera*, to be made out.

Up to this stage sections show no vitellophag cells imbedded in the yolk, but slightly later the immigration of a considerable number of cells of the posterior mesendodermal region takes place, and at about this same time a stout, backwardly projecting process of the mesendodermal region became visible, and this I take to be the liver endoderm, since it is closely comparable to the process described in *Jaera* as the Anlage of the liver lobes. Its origin I have not been able to make out, nor is its fate at all certain, since it quickly disappears, most probably becoming indistinguishable from the mesoderm cells.

In Fig. 37 is represented a somewhat later stage of development, in which it is seen that the teloblasts have increased considerably in number, there being now twenty-two of these cells, ten on one side of the center one (*cT*) and eleven on the other, a disparity of number on the two sides, which is repeated in the specimen from which Fig. 38 was drawn. The source of the teloblasts indistinguishable in the previously described stage (Fig. 36) I have not been able to discover. They do not, however, appear to be formed by the transverse division of the eleven primary teloblasts, and it seems probable that they are due to the addition to the teloblast row of cells lying originally lateral to it, or of the progeny of such cells. Whatever may be the origin of these cells the teloblast row now forms almost a semicircle, enclosing a mass of cells which represent the mesendoderm. In front and at the sides of the teloblasts are to be seen the teloblastic rows, those on either side of the middle line being a little longer than those further towards the sides, a point which is not well brought out in the figure, it being difficult in it to distinguish between the anterior teloblasts and the most posterior cells of the naupliar region.

These latter, and the cells in front of them, are characterized by their irregular and scattered grouping, so that they present a marked contrast to the regular rows of the meta-naupliar region. The more anterior cells, however, are more closely aggregated, and form the so-called cephalic lobes of the embryo.

These, and the cells intervening between them and the anterior teloblastic cells, form the lateral ventral bands, which have approximated much more closely to each other than in earlier stages. No transverse band, such as was found in *Jaera*, can be made out in this stage (its absence in earlier stages has already been noticed), unless a patch of cells (*DO?*) lying in the middle line between the two cephalic lobes is its representative. This is possible, though another possibility, that this patch represents a rudimentary dorsal organ, should not be lost sight of. In the embryo figured another patch of cells, which stained quite deeply, was found in the median line immediately in front of the teloblastic cells; I cannot discover any special significance for these cells, which are indistinguishable in later stages, and regard them simply as naupliar cells of the midline joining the two lateral bands.

In the stage represented in Fig. 38 the teloblastic rows have increased considerably in length, the rows originating from the eleven primary teloblasts being longer than any of the others, which decrease in length the further they lie from the median line (compare in this respect *Jaera*, Figs. 25 and 26). The teloblast row no longer forms a semicircle as in the previous stage, but is now straightened out, indeed, is slightly concave anteriorly. It has at the same time extended backwards over the region previously occupied by the mes-endoderm, which layer is unrepresented in the drawing, and it may be noted, too, that all the teloblastic rows are parallel to each other, and have an antero-posterior direction. The more lateral ones have not increased in length very much when compared with the previous stage, but the more median ones have, and it would seem that the straightening out of the teloblastic row was due to this excessive growth of the more median rows, which are consequently pushed backwards, the lateral teloblasts remaining fixed or being pushed outwards only. This explanation of the change of form of the teloblast row was suggested in the description of *Jaera*, but the evidence in its favor is much more pronounced in the present species. Special attention may be directed to the median teloblastic row, the cells of which are markedly different from those of the other rows, and constitute,

as in *Jaera*, the Mittelstrang. Behind the teloblasts is a mass of ectoderm cells more or less irregularly arranged, which represent the hind end of the body, and among which the anus will form.

With regard to the naupliar portion of the embryo at this stage, there are but few points to be noted. The separation between the naupliar and post-naupliar regions is clearly indicated by the scattered arrangement of the cells, and the lateral ventral bands have approximated still further than in Fig. 37. On each side, just at the junction of the two regions, is to be seen a small patch of cells which constitutes the Anlage of the lobed (lappen-förmige) organ of *Asellus*.

3. *The Segmentation and Formation of the Germ-layers in Porcellio and Armadillidium.*

The similarity between the ova of *Porcellio* and *Armadillidium* in their development is so great that the two forms may be considered together.

Porcellio has been the subject of several papers, which present most remarkable discrepancies in the descriptions given of the segmentation, and, in considering them, papers on *Oniscus* may also be included, since my own observations on forms belonging to this genus and on the nearly related genus *Philoscia*, though most fragmentary, nevertheless clearly show that the type of segmentation is identical with that of *Porcellio*. The earliest paper is by Bobretzsky ('74) on *Oniscus murarius*, and the results recorded in it have been accepted and copied into many general works. According to Bobretzky the segmentation of *Oniscus* is of the epibolic variety, and resembles that described by Van Beneden ('69) for *Mysis*. According to his account a quantity of protoplasm collects at one pole of the egg, forming a colorless, transparent, rounded mass, which, in the next stage observed, is represented by a plate or disc of cells lying sometimes at the pole of the egg, sometimes near the pole, and sometimes upon the side. In later stages this disc extends over the yolk, the mes-endoderm forming from it before it reaches the equator in cases where it originally occu-

pied a polar position. It will be seen that there is a large gap in Bobretzsky's observations between his supposed earlier stage and the next he describes, and I believe from my observations on an undetermined species of *Oniscus* that he is entirely in error, that his disc represents a comparatively late stage of segmentation, and that his first stage was an abnormal egg. In other words, the *Oniscus* which I observed had a typical centrolecithal segmentation closely similar to that of *Asellus*, certain of the cells aggregating at an early stage to form a blastoderm or disc, the rest of the yolk being covered by scattered stellate cells whose existence Bobretzsky probably overlooked. Nusbaum ('86), who also studied *Oniscus murarius*, did not observe the segmentation stages, but states that, in ova in which the disc was not yet present, the central portion of the yolk stained deeply and was coarsely granular, and seems inclined to regard this as indicating the presence at that point of the "plasme formatif," though he was unable to distinguish the first segmentation nucleus.

Close on the heels of Nusbaum's paper appeared one by Reinhard ('87) on *Porcellio scaber*, for which he described a typical centrolecithal segmentation, the cells formed by the division of the original centrally situated segmentation cell gradually migrating towards the surface of the yolk, upon which they are scattered, forming what Reinhard terms "Inselchen." Up to this point this author's observations tally perfectly with my own, but his account of the formation of the mes-endoderm is, I think, incorrect. He says: "Unter diesen Inselchen können sich an verschiedenen Stellen mehrere Lagen von Zellen befinden . . . Der Raum zwischen den Inselchen füllt sich allmählich an, und bald zeigt sich ein Theil der Eioberfläche mit einer dichten Lage des Ectoblasts bedeckt. Die unter dem Ectoblast liegenden noch indifferenten Zellen stellen das primäre Entoderm dar. Nur allmählich differenziren sich dieselben in Mesoderm- und Entoderm-Zellen." In how far my results differ from these will be seen later.

Roule has contributed several short notices on the development of the Isopods, and especially of *Porcellio*. In the first of these ('89) he describes the egg of *Porcellio* as having at

one end a patch of hyaline plasma which segments into large cells extending over the surface of the yolk by the addition of new material added from the yolk. So far as I can understand the brief notice, he believes the mesendoderm to form very much in the manner described by Reinhard. He speaks, however, of nuclei which "naissent spontanément" (!) at the periphery of the vitellus, the yolk particles in their neighborhood assuming an altered appearance. In a general way he agrees with Bobretzsky regarding the segmentation, and with Reinhard in the formation of the germ layers, unfortunately being in error with regard to both processes. In a second paper (Roule, '90) the same author evidently mistakes an egg in a somewhat advanced stage of segmentation for one which has not yet divided. He describes the occurrence of islands of formative plasma at the surface of the yolk, but asserts that only one of them, situated at one pole of the egg, contains a nucleus! This nucleated island divides and forms a disc which gradually extends and fuses with the non-nucleated islands, which do not divide until the migration (!) into them of nuclei or particles of nuclear matter from the disc, and the extension of the latter is thus continued. The disc also increases in thickness by the deutoplasm in its vicinity assuming the character of formative plasm, into which nuclei migrate from the disc cells. A truly remarkable series of phenomena! In this paper, however, he corrects one gross error of his earlier one, denying the spontaneous formation of nuclei, and on the whole comes a little nearer to the truth, though still almost hopelessly in error. His third paper (Roule, '91) deals with the formation of the germ layers, and he advances the idea that the endoderm, *i.e.*, the liver endoderm, appears in place as the result of a proliferation of the blastoderm cells on each side of the body a short distance behind the head, while the mesoderm arises from the proliferation of the blastoderm cells at various regions, practically, indeed, in every region, a view which is emphasized in later contributions (Roule, '92, '92[a]). Roule's statements are evidently assumptions based upon imperfect observations, and if proper use had been made of surface views, many of them would never have been published.

It is evident that considerable difference of opinion exists as to the mode of segmentation and the development of the germ layers in the Oniscidæ. I have not been able to follow out the development of *Porcellio* and *Armadillidium* quite so thoroughly as that of *Asellus* or *Jaera*, but my observations are sufficient to allow of a close comparison with these forms. The fertilized but unsegmented egg of *Porcellio* or *Armadillidium* resembles very closely that of *Asellus*, though considerably larger. It is enclosed in a chorion and a vitelline membrane, and the surface of the yolk is covered by a thin layer of protoplasm, while in the center is an amoeboid mass of protoplasm surrounding the first segmentation nucleus (Pl. VII, Fig. 39). In the next stage (Fig. 40) two such amoeboid cells are to be found in the egg, which, it may be here remarked, is much more irregular in shape than either *Asellus* or *Jaera*, orientation being accordingly much more difficult than in these forms. This 2-celled stage is succeeded by one in which there are four cells (Fig. 41), the arrangement being practically the same as that described in *Jaera;* for instance, the line joining two of the cells (*A* and *B*) being at right angles or nearly so with that joining the other two (*C* and *D*). I was not able to obtain ova which showed the original two cells in the spindle stage, so cannot state how the arrangement is produced, or even that the cells are correctly identified with those of *Jaera;* they are identical in position, but whether they are also genetically identical I cannot state. One further point in connection with this stage deserves mention. The peripheral protoplasm, which in earlier stages was uniformly distributed over the yolk, at this stage becomes concentrated to a certain extent at one region of the egg (*pp*), a region which will later become the posterior end of the naupliar portion of the embryo, and may be regarded, therefore, as the posterior pole of the egg. This aggregation of the peripheral protoplasm is an exceedingly interesting process, marking out as it does thus early a polar differentiation of the egg, and giving also a suggestion as to one of the possible explanations for the belief in an epibolic segmentation in *Porcellio*. In order that there may be no question as to the centrolecithal character of the segmentation, I represent (Fig.

42) a section through an ovum in the four-celled stage. The section has passed through two of the cells, and shows clearly their position in the yolk exactly as in *Jaera* or *Asellus*, or any other form which segments in the typical centrolecithal manner.

In the eight-celled stage there seems to be considerable irregularity in the arrangement of the cells in different ova, but in some an arrangement comparable to that of *Jaera* was found. In Fig. 43 is represented an ovum of *Armadillidium* which is just passing into the 16-celled stage, all the nuclei but one being in the spindle stage. This one, which has not yet started to divide, lies near the posterior pole of the egg and corresponds to the *D* cell of *Jaera* and *Asellus* so far as its position is concerned. In other eggs both of *Porcellio* and *Armadillidium* this same arrangement was found, and it seems to be normal for some eggs, though in others it could not be made out. The 16-celled stage derived from an egg of this arrangement shows as in *Jaera* two cells at the posterior pole (Fig. 44, *D* and *X*), surrounded by a circle of apparently four cells in *Porcellio* (Fig. 44) and five in *Armadillidium* (Fig. 45). Whether this difference be due to the slightly different stages represented, the *Porcellio* ovum represented having just completed division, while that of *Armadillidium* is just passing into the 32-celled stage, I cannot say, but just as the number of cells composing the circle differ in *Jaera* and *Asellus*, so too they may do here without any appreciable alteration of the similarity of the general features of development. In fact I may state that in one egg of *Porcellio* I saw six cells in a circle, and I believe that even in different eggs from the same individual there may be considerable differences of arrangement of the spherules at this stage, although the two polar cells are probably always distinguishable. Sections of ova of *Porcellio* in this stage (Fig. 47) show that the cells have reached the surface of the yolk and are imbedded in the peripheral protoplasm, so that the egg is a syncytial blastula whose cavity is completely filled with yolk, there being certainly no nuclei, and apparently no protoplasm, scattered among the yolk granules.

The 32-celled stage of *Porcellio* I have not seen, but in *Armadillidium* (Fig. 46) the two polar cells have divided so as to form four cells arranged as shown in the figure, and around them is a circle of eight cells whose origin I have not made out. The appearance of the ova at this stage is markedly different from that of *Jaera*, and presumably *Asellus* also, on account of the total absence of superficial segmentation lines. Indeed, in neither *Porcellio* nor *Armadillidium* does a segmentation of the yolk ever occur, a condition no doubt explained by the greater amount of yolk presented in these forms. It was probably some such stage as this which Roule mistook for an unsegmented ovum.

The next stage which I have observed corresponds to the 64-celled stage of *Asellus*, though whether there are actually sixty-four cells present I cannot state. The most striking feature of the stage (Fig. 48) is the commencement of a concentration of a number of the cells around a point which in some cases is on the side of the egg, and in others at its extremity. The concentration is indeed but feebly marked at this stage, but is nevertheless indicated, and becomes pronounced in the next stage. In the center of the region around which the concentration is taking place *four* cells (D^1–D^4) may be observed which differ somewhat from the others in their staining properties. These may be the four polar cells of the preceding stage or else four of the eight cells which would be produced from their division. Which of these alternatives is correct I cannot state, but from analogy with what occurs in *Asellus* I am inclined to believe the second one the more probable. These cells give rise to the mes-endoderm, and in the next stage (Fig. 49) have increased to eight in number, the concentration of the other cells increasing. These last two figures (Figs. 48 and 49) represent ova of *Porcellio;* I did not find *Armadillidium* ova in corresponding stages, but the next figure (Fig. 50) represents an egg of that genus in a slightly later stage. The mes-endoderm is formed of a much larger number of cells, and the concentration of the ectodermal cells around it is very evident. In Figs. 51 and 52 slightly later stages of *Armadillidium* are shown, presenting a still greater

increase in the number of mes-endoderm cells as well as of the surrounding ectoderm cells, the latter increase being due mainly, if not entirely, to the division of the cells which have already undergone concentration in Fig. 50, there being probably no further migration of cells from the dorsal surface of the egg to form the blastoderm. In these last two figures a row of four cells (*Me*) not at all well-marked off, however, lies at the anterior margin of the mes-endoderm zone, and it is possible that they correspond to the row of mesoderm cells occurring in *Asellus*, though it has been impossible to distinguish them in later stages, and accordingly their significance is uncertain.

An egg of *Porcellio*, in a slightly later stage than Fig. 50 though earlier than Fig. 51, is represented in Fig. 53. From this it will be seen that the process of development proceeds in this species as in *Armadillidium*. There is a similar continuation of the concentration of ectoderm cells around the mes-endoderm, to form the blastoderm, and in Fig. 54 a later stage is shown, in which the cells of the ectodermal portion of the blastoderm are considerably increased, and in which the concentration is somewhat greater. In the preparation from which this drawing was made the blastoderm had been dissected from the surface of the yolk, and in the operation was slightly torn; enough of the yolk was left adherent, however, to support a few of the scattered ectoderm cells which do not take part in the formation of the blastoderm. Up to about this stage in *Porcellio*, and in *Armadillidium* also, all the cells are at the surface of the yolk, but now careful focusing of the mes-endoderm region shows that there are a number of cells below the surface, a fact which is also shown by sections of the blastoderm (Fig. 55). I found no indication of spindles perpendicular to the surface in my preparations of this stage, and am inclined to believe that the cells of the lower layer arise by immigration rather than delamination, although I am not in a position to maintain that the latter process does not occur. It is certain, however, that at this stage lower layer cells occur only in the mes-endoderm region.

In Fig. 56 a somewhat older blastoderm of *Porcellio* is shown, similar preparations of *Armadillidium* having also been ob-

tained. Here the region occupied by the mes-endoderm has greatly increased, being represented in the figure by the shaded area, though it is probable that a portion of the area really lies outside the true mesendodermal region, the anterior margin of the area appearing dark in the preparation on account of the lower layer mesendodermal cells having begun to migrate forwards and to scatter beneath the general surface of the blastoderm. This forward migration and scattering has proceeded further in Fig. 57, which is from *Armadillidium*, though preparations similiar to this and the succeeding figure were obtained from *Porcellio*. The concentration of the cells to form the blastoderm has now reached its greatest extent, the nuclei are crowded together, and the entire area covered by the blastoderm, notwithstanding the increased number of cells, is little if any greater than that which it covers in the stage represented in Fig. 53. A little behind the middle of the blastoderm is seen a very dark area (*MEn*) which represents the mesendodermal region in which the lower layer cells are heaped together to form a mesendodermal plug projecting into the yolk, and anteriorly, as well as posteriorly, less dark areas are to be seen, which are due to lower layer or mes-endoderm cells which have migrated forwards and backwards from the mes-endoderm region. I say migrated, because I have not been able to find any evidence for the assumption that they originate *in situ* by delamination from the ectodermal portion of the blastoderm. Numerous spindles are to be found in blastoderms of this stage, but I have never seen any directed perpendicularly to the surface, and believe that here as in *Jaera* and *Asellus* all the mes-endoderm comes from the cells of the mesendodermal region.

In this figure there is to be seen just at the front edge of the mesendodermal region an imperfectly defined row of cells which are the first indication of the ectodermal teloblasts (*T*). They are not sufficiently well marked to determine their exact number, though they seem to be more numerous than eleven, which was the number first differentiated in *Asellus*. They serve to mark accurately the anterior edge of the mesendodermal region.

The scattering of the mesendodermal cells and the more perfect differentiation of the ectodermal teloblasts continues in later stages, producing the appearance shown in Fig. 58. Here the entire area of the blastoderm presents a dark appearance due to the presence under the ectoderm cells of lower layer cells, the mesendodermal region being but little darker than the remaining portions. The scattering of the mesendodermal cells is complete; and careful focusing, as well as sections, show the presence of vitellophag cells in the yolk. No definite differentiation of these cells from the remaining mesendodermal elements is, however, to be distinguished, and, taking the three forms of cleavage here described into comparison, it will be seen that we have in them three gradations of differentiation of the mesendodermal elements. In *Jaera* there is first of all a distinct differentiation of the vitellophags from the rest of the mes-endoderm and later a differentiation of the liver-endoderm for the mesoderm, so that three distinct portions of mes-endoderm are visible. In *Asellus* the differentiation of vitellophags from the remaining mes-endoderm is at certain stages distinct, though later becoming inconspicuous, and a recognizable differentiation of liver-endoderm is questionable. Finally, in *Armadillidium* and *Porcellio* no differentiation into the three portions can be made out, and it is necessary to speak simply of the mes-endoderm. There can be no doubt, however, that this mes-endoderm is equivalent to the mesoderm, liver-endoderm, and vitellophags of *Jaera*, giving rise to cells which play the parts taken by these various elements in the development of the embryo. The significance of this variation in the differentiation of the mesendodermal elements will be considered more in detail in a later portion of this paper.

In the stages represented in Fig. 58 the further differentiation of the ectodermal teloblasts is also shown, there being thirteen recognizable in the preparation figured. One cell of the row is hardly on a level with the others, and one is tempted to consider it the central teloblast, in which case there is a marked disparity in the number of cells present on either side of it, there being but five on one side and seven on the other. The number five is significant in view of what has been de-

scribed as occurring in *Asellus*, but there seems to be less definiteness in the number of the cells composing the teloblastic row when it is first recognizable than in that form.

The development of *Porcellio* and *Armadillidium* has now been carried to a stage in which the germ-layers may be said to be differentiated, though as already indicated it is as yet impossible to distinguish the liver-endoderm from the mesoderm. This differentiation, as is essentially the case with *Asellus*, only supervenes at a much later stage, and a halt may be conveniently called here. In order, however, to carry the account up to the stage at which it was left in *Asellus*, an additional figure (Fig. 59) is given, in which the embryo is distinctly recognizable. The figure was drawn from an ovum viewed by direct illumination as an opaque object, and shows the optic lobes distinctly indicated. Posteriorly are a number of scattered cells, in front of which is the row of ectodermal teloblasts (*T*), which are smaller in comparison with the cells of the teloblastic rows (*Tr*) to which they have given rise than is the case in *Asellus*. There are about twenty-three teloblasts in the preparation figured, and it is noticeable that there is no indication of the existence of eleven primary teloblasts such as occurred in *Asellus* and probably also in *Jaera*, the number of cells in the most anterior of the four teloblastic rows which are present being but slightly less than that of the posterior row. This is in harmony with the indefiniteness of the number of cells composing the row in earlier stages, and it may be supposed that in *Armadillidium* and *Porcellio*, the teloblasts do not enter upon their characteristic method of division until nearly the entire row has differentiated. No difference can be distinguished between the central and the remaining teloblasts, nor does the median teloblastic row of cells differ from the remaining rows; and furthermore it seems that in the species under consideration the scattering of the mes-endoderm takes place relatively earlier in comparison with the formation of the teloblastic rows than in either *Asellus* or *Jaera*, since in the stage figured there is no indication of any special mass of mes-endoderm remaining in what was originally the mes-endoderm region, but the scattering has been complete. In the naupliar region of the

embryo certain definite dark patches occur, indicating the presence of special masses of lower layer cells, which mark out imperfectly the naupliar somites. Finally it is to be noticed that in front of the embryonic region proper there is a dark patch (*DO?*) situated in the mid-line, which recalls the similarly situated patch occurring in *Asellus* (Fig. 37, *DO?*) and may represent the dorsal organ.

As was stated at the beginning of the paper, I have had for study a certain amount of material representing stages in the development of *Cymothoa* and *Ligia*, but I have not considered it necessary to devote space to a special description of the results obtained from it, both on account of the incompleteness of the material and the general similarity which exists, so far as the material went, between the development of these forms and that of the species just described. *Cymothoa* has been the subject of a paper by Bullar ('78), and more recently Nusbaum ('93) has published an account of the development of *Ligia oceanica*, unfortunately, however, concealing his results under cover of the Polish language, the majority of embryologists being obliged to rely, for information as to the contents of the paper, partly on the brief abstract of it which appeared in the *Biologisches Centralblatt*, and partly on the preliminary notice published by its author in the same journal ('91).

In the earliest stage of *Cymothoa* which I possess the development has reached a stage which corresponds in all essentials to that of *Porcellio* represented in Fig. 56, and the later stages up to the formation of the embryo likewise resemble what has been described for *Armadillidium* in all essential particulars. Thus there is the same absence of differentiation of the mesendoderm, the same early scattering of the mesendodermal elements, and apparently the same absence of a distinct differentiation of eleven primary ectodermal teloblasts. In fact the preparations are so similar to those of *Porcellio* and *Armadillidium* that I have not considered it necessary to figure them. The earliest stage I possess is identical in age with that figured by Bullar, and like that author I cannot make any conclusive statements as to the nature of the segmentation. This much, however, is certain, that there occur scattered over the surface

of the yolk at this stage numerous cells just as in the forms whose segmentation I have described, and it seems probable that in *Cymothoa* the segmentation follows the centrolecithal type, notwithstanding the large amount of yolk which is present, the egg measuring about a millimetre in length with a diameter but slightly less.

In *Ligia occanica* Nusbaum apparently believes that the segmentation is of the discoblastic type, and Van Beneden [1] maintains the same view. So far as Nusbaum's observations are concerned it must be pointed out that the material at his disposal was hardly sufficiently representative of the earliest stages to allow of perfect certainty on this point, and in view of what certainly occurs in other Oniscidæ a certain amount of doubt as to the accuracy of his interpretation of the observed phenomena is legitimate. My material, unfortunately, is likewise too imperfect to settle the question, though the earliest stage I have serves to emphasize the doubt. A section through an egg of this stage is represented in Fig. 60, and shows distinctly four nuclei and a fragment of a fifth, the protoplasmic masses in which they are imbedded lying flush with the surface of the yolk. In a surface view of the same egg before it was imbedded the nuclei were seen to be evenly scattered over the surface, and in running through the series of sections, which was complete, over eighty nuclei were counted. There were none whatever in the interior of the yolk, and none of the cells had separated from the yolk any more than those figured. If the segmentation had been discoblastic one would expect to find some of the cells more or less aggregated at one region of the egg but this does not occur, and the appearance presented is identical with that found at a somewhat earlier stage of development in *Porcellio*. Further observation is necessary, I believe, before it can be definitely decided whether the segmentation of *Ligia* is discoblastic or superficial, *i.e.*, centrolecithal.

[1] Van Beneden's paper, "Recherches sur la composition et signification de l'œuf," published in the *Mém. de l'Acad. roy. de Belgique*, T. xxxiv, 1870, I have not seen, the above statement being made on the authority of Korschelt and Heider ('91).

The next stage which I have seen is one apparently slightly younger than that of which Nusbaum has represented sections in Figs. 18 and 20 of his Polish paper. In surface view there is seen to be a distinct blastoderm which resembles closely the stage of *Porcellio* shown in Fig. 54, except that a greater number of cells enter into the composition of the blastoderm, a fact probably explained by the greater amount of yolk present in the egg of *Ligia*. Certain of the cells of the dark area, *i.e.*, according to my interpretation the mesendodermal area, have already sunk beneath the surface, and in the center of the area is a distinct depression. Other material of the blastodermic stage which I possess shows but little difference from this, the cells in some of the ova examined being somewhat more numerous, and between this condition and one in which the embryo is distinctly outlined there is a gap. Nusbaum ('93) has represented two later blastodermic stages in his Figs. 1 and 2, showing in addition to a central thickening two lateral ones, and assumes that the former gives rise to the endoderm and the latter to the mesoderm. Sections which he gives, repeated in the abstract, show that in the region of the lateral thickenings lower layer cells occur, and he believes that they have been produced *in situ*. For this belief, however, it seems to me he has given no conclusive proof, and it is quite possible that we have to do here, as in other Isopods, with a migration forwards of mesoderm cells, both the endoderm and mesoderm differentiating from the central dark area of the blastoderm and from this alone. I do not assert that this is so, but the fact of the close resemblance of the two stages I have figured with what I have found in *Porcellio* and *Armadillidium* suggests the supposition that all the processes of development of *Ligia* resemble those of *Porcellio*.

4. *General Consideration of the Segmentation.*

In the ova of the four species whose development has been thoroughly studied we have to do with typical cases of centrolecithal or superficial segmentation, and certain facts have been described which I believe have important bearing upon

some of the problems which have of late been exciting no little attention from embryologists. Before passing on to consider these facts, however, I wish again to emphasize what has already been said as to the syncytial nature of the developing egg up to the stage at which the cells completely separate from the yolk, since it is to the existence of the syncytium that the interest which attaches to the centrolecithal segmentation as seen in the Isopods is due. In dealing with holoblastic ova in which there is apparently a formation of distinct spherules, embryologists have been too apt to concentrate their attention on the individual spherules, and to regard them as more or less independent units and not as parts of a continuous whole, and it is largely to the assumption of this standpoint that the mosaic theory of development owes its existence. There is, however, an increasing tendency towards the rejection of this theory and towards a return to the view long ago (1867) enunciated by the botanist Hofmeister, according to which the growth of the individual cells is determined by the growth of the entire organism, an idea admirably expressed by DeBary in the aphorism "Die Pflanze bildet Zellen, nicht die Zelle bildet Pflanzen." Rauber ('83) in an exceedingly suggestive paper extended this idea to animals; Heitzmann has carried it to its full limits, and to-day it is upheld by such authorities as O. Hertwig ('92), Whitman ('94), and to a certain extent, Wilson ('93).

In developing holoblastic and meroblastic ova it is difficult to demonstrate actual continuity of protoplasm throughout the various spherules, and so far as they are concerned the idea just referred to rests upon a theoretical basis. With centrolecithal ova the case is different, and it is not difficult in the ova of the species I have described to demonstrate the existence of a syncytium. It is not improbable that such a condition will be found in all centrolecithal crustacean ova, though up to the present, so far as I am aware, it has been described by a single author only, namely Samassa ('93) in his paper on the development of the Cladoceran *Daphnella*. In this form, as well as in the Isopods I have described, a considerable amount of yolk is present, and there is no indication of total cleavage. There

are, however, numerous Crustacean ova in which such a form of cleavage does occur, as, for instance, those of *Lucifer* (Brooks, '83), and between the holoblastic cleavage of this form and a typical centrolecithal cleavage, numerous gradations are to be found (see Korschelt and Heider, '91). The exact character of the cleavage of the ova of the primitive Crustacea is of course a matter of speculation, but the fact that centrolecithal cleavage seems to be characteristic of the Crustacea, occurring in practically all the groups in some grade or other, this fact indicates that cases such as those of *Lucifer* are secondary. If it be true, then, as I suppose, that all typically centrolecithal ova are syncytia in the early stages of development, are we to believe that with the loss of yolk and the assumption of a holoblastic cleavage, all direct continuity between the spherules is dissolved? Are we to believe that there is no continuity in *Lucifer*, notwithstanding that in all probability there was continuity in the ova of its ancestors?

In this connection the ovum of *Peripatus capensis* is of no little interest. It has been pointed out by Sedgwick ('86) that the ova of *P. Novae Zealandiae*, *P. capensis* and *P. Edwardsii* form a series, so far as the amount of yolk which they contain is concerned, the first named having a considerable amount while the last has practically none at all. Now, according to the statements of Miss Sheldon ('88) the ovum of *P. Novae Zealandiae* undergoes a segmentation which is essentially centrolecithal and forms a syncytium, while in *P. capensis* the cleavage approaches the holoblastic form and yet a syncytium again results. The spongy character of the *capensis* ovum strongly suggests that the ancestors of that form possessed yolk-laden ova, and that the loss of the yolk has been comparatively recent. This loss has not, however, resulted in the dissolution of the continuity of the spherules, and furnishes some support for the supposition that, even in such cases as *Lucifer*, there may be also a continuity of protoplasm, the separation into distinct spherules being only apparent.

In the ova of many Decapod Crustacea the formation of what are termed yolk pyramids occurs, and it has generally been supposed that the various pyramids were separated by

distinct "segmentation planes." In the Isopods there is no very distinct formation of yolk pyramids, though the appearance of segmentation planes upon the surface of the yolk in the 32-celled stage for instance of *Jaera*, is the equivalent of the process. Physiologically, the occurrence of the yolk pyramids is exceedingly interesting, but morphologically it has no further significance than is to be found in typical cleavage. I wish, however, to refer here to the appearance presented in section by ova of *Jaera* in which the yolk cleavage has occurred. In Fig. 20 such a section is represented, and from it the cause of the appearance of the yolk cleavage can be clearly seen. Below the large mes-endoderm cells (*MEn*) can be seen a distinct line of protoplasm, also indicated beneath the ectoderm cells (*Ec*). This line corresponds, when it reaches the surface, with the lines of yolk cleavage, and there is no reasonable room for doubt but that it is the presence of this line which produces the appearance of the cleavage. The line, however, is really a section of a membrane which behaves to reagents in the same way as the general protoplasm and in addition is connected with this by branching protoplasmic filaments. Furthermore, and this is an important point, processes run off from the membrane centrally into the yolk, undoubtedly uniting with similar processes from other cells and producing a protoplasmic network throughout a portion of the yolk outside the limits of the membrane. This membrane cannot, I believe, be regarded as a thin membrane of dead material, *i.e.*, the ordinary conception of a cell wall cannot be applied to it ; it is in reality living protoplasm and consequently it is evident that *the cleavage of the yolk does not interrupt the protoplasmic continuity*. Whether or not this idea applies also to cases such as *Astacus*, in which there is a formation of typical yolk pyramids, remains to be seen.

For some time after the cells have reached the surface of the yolk, their stellate outline indicates the continued existence of the syncytium, but later they appear to round off and actual continuity cannot be demonstrated. This change of appearance can be seen by comparing Fig. 48 or 49 of *Porcellio* with Fig. 53, which shows a later stage in the development of the

same species. To what extent this apparent discontinuity of the protoplasm of the various cells is a reality, and how far it is of importance for the complete histological differentiation of the cells I do not propose to discuss, but wish to point out that the *existence of a syncytium is no bar to a certain amount of differentiation.* Thus to confine our attention to *Jaera*, in which early differentiation is most marked, in the eight-celled stage the cell *D* has been separated off and, as has been seen, gives rise solely to vitellophags, and at the sixteen-celled stage the ectoderm is thoroughly differentiated from the mes-endoderm. In this stage, however, there is no visible difference in the cells, and that there is a differentiation can only be determined by tracing their future history. In the next stage, however, which, as has been shown, is still a syncytium, histological differentiation is distinct, the vitellophags presenting a very different appearance from the mes-endoderm cells, and these from the ectoderm.

This syncytial differentiation reminds one very forcibly of the differentiation found in the ciliate Infusoria ; it is a differentiation which proceeds independently of the existence of cell boundaries, the force which compels it being at present beyond our ken, and not to be regarded, it seems to me, as resident in the nucleus. The fact of the occurrence of cytoplasmic differentiation in uninuclear Infusoria stands in opposition to any such view, and a phenomenon which has been described on a preceding page as occurring in the early stages of development of *Porcellio* and *Armadillidium* seems to demonstrate that cytoplasmic differentiation may occur independently of definite nuclear influence. I refer to the remarkable concentration of the peripheral protoplasm which occurs in ova of these forms at the four-celled stage. (See Figs. 41–44.) The region towards which the concentration takes place is that in which later the blastoderm will be developed and at the time of its appearance the nuclei present in the ovum are still some distance from the surface, embedded in the yolk, and only in the 32-celled stage do they pass into the peripheral protoplasm, the cytoplasm which surrounds them fusing with it. We have to do here with *a precocious segregation of a portion of the cytoplasm which*

is to take part in the formation of the blastoderm, and this segregation supervenes not in accordance with any previous location of a nucleus, but independently. I do not of course mean to assert that the nuclei may not possess a coördinating or even a trophic action upon the cytoplasm, but that they are directly responsible for the segregation or concentration seems to me an unwarranted assumption. The phenomenon stands apparently in relation to the growth of the entire organism rather than to that of a part of it, and is an instance from the animal kingdom indicating the distinction so forcibly pointed out by Sachs ('87) as existing between growth and cell division.

It has already been pointed out (see p. 74) that in the four-celled stage of *Jaera* there is an arrangement of the spherules, apparently comparable with that found in holoblastic ova which undergo a "spiral" cleavage. In later stages, however, the cleavage departs widely from the typical mode of progress of the spiral method, probably in harmony with the precocious differentiation which has already been shown to exist. The Isopod segmentation agrees strictly with none of the three types of cleavage defined by Wilson ('93), though it bears most resemblance, in early stages at least, to the "spiral" method, and the peculiarities of later stages are to be ascribed to the same causes as Wilson proposes in explanation of the bilateral type. In his earlier discussion of the forms of cleavage ('92) Wilson held that the spiral type of cleavage was determined by the spherules having a tendency to arrange themselves along the lines of least resistance, assuming therefore a purely mechanical cause acting from the exterior as an explanation. In his later paper, however, he modifies this extreme view, stating his opinion that "*cleavage forms are not determined by mechanical conditions alone.*" Assuming that by "mechanical conditions" he means conditions extrinsic to the ovum, I believe that in *Jaera* we have practically a demonstration of the correctness of this view.

Let us briefly recall the rearrangement of the protoplasm which obtains in *Jaera*. Completely surrounding the exterior of the ovum is a thin layer of peripheral protoplasm, corresponding to the "Keimhautblastem" of the egg of Insects, and

within this is the yolk scattered in the meshes of a protoplasmic network extending from the peripheral protoplasm to a more or less centrally situated mass containing the nucleus, this central mass and the nucleus alone undergoing cleavage, at least in the early stages. As cleavage proceeds the central masses formed by it separate from one another, remaining connected, however, by strands of the network. It is difficult, therefore, to understand how any extrinsic forces can determine the position assumed by the central masses, and since they are not in contact with one another, but are suspended, as it were, in a protoplasmic reticulum, like knots in a fish-net, it is also difficult to understand how Berthold's ('86) law of minimal contact areas can determine their position. I see no escape from the conclusion that *the cleavage form of Jaera is determined entirely by intrinsic conditions*, and though we cannot exclude the action of extrinsic forces in holoblastic ova, yet the presumption is allowable that even in these the intrinsic forces are important. In discussing the cause of the direction of cell division, it must be remembered that the forces which primarily determine karyokinesis reside in the cell, since recent observations have strengthened the view which regards the archoplasm and the centrosomes as of the greatest importance in this respect. The generalization of O. Hertwig that the spindle tends to form in the direction of least pressure is one to which there are exceptions, and in centrolecithal ova, such as those of the Isopods, it cannot come into the question. We are left, then, no choice but to refer the *vis essentialis* which determines the direction of the karyokinetic spindle, and therefore the cleavage form of *Jaera*, to *the constitutional peculiarity of the ovum.*

I do not, however, in the least intend to imply that external conditions, such as pressure, *etc.*, do not influence the direction of the spindle in holoblastic ova ; indeed, the evidence we have indicates that they do ; my remarks refer simply to the ova of *Jaera*, and those of similarly developing forms. It may be pointed out, however, that the formation of the second polar globules, if not of the first also, is apparently determined by intrinsic forces, and the relation of the first segmentation plane to the point of emergence of the polar globules is also probably

due to these same forces. We must assume, accordingly, that intrinsic forces reside in all ova, though they may be overshadowed by external influences in some cases. In fact this idea may be carried further, and it may possibly be that the action of external forces may be sufficient to interfere with the arrangement of the cells which would result if they were excluded, and the histological differentiation of the ovum be retarded thereby.

One other point which the study of Isopod cleavage has suggested seems to be worthy of notice here. It is connected with the question of the value of cytogeny as a basis for homology, a question which has already been discussed by Wilson ('92), who points out that "cells having precisely the same origin in the cleavage, occupying the same position in the embryo, and placed under the same mechanical conditions, may nevertheless differ fundamentally in morphological significance." A comparison of the cleavage of *Jaera* with that of *Asellus* brings support to this view. Thus in the eight-celled stage of the former species (Fig. 9) we find at one pole of the egg a cell *D*, which gives origin to all the vitellophags of later stages; in the eight-celled stage of *Asellus* (Fig. 30) we find an exactly comparable arrangement of the cells, but the cell *D* has by no means the same morphological significance, inasmuch as it does not contain vitellophag elements alone. Similarly in *Jaera*, in the sixteen-celled stage (Fig. 12), there are six cells, A', c, c', and d', which give rise to the liver-endoderm and mesoderm of later stages, while in *Asellus* it will be seen that the same cells, the same, that is, so far as their cytogeny is concerned, are entirely ectodermal. Indeed the difference between the two species may be expressed by stating that the differentiation of the germ-layers takes place practically at one stage later in the cleavage in *Asellus* than in *Jaera*.

The significance of this is by no means certain, though it is interesting to note that an explanation of it may be found in the different size of the two ova. Thus the egg of *Asellus* is several times the size of that of *Jaera*, and it may be that the extra amount of yolk acts in retarding the differentiation, or, in other words, a certain spatial relationship of the blastula cells

may be necessary before differentiation can take place, this relationship, on account of the greater quantity of yolk in *Asellus*, supervening later in that form than in *Jaera*. This suggestion is tempting, but it must be noted that a further retardation of the differentiation does not take place in *Armadillidium* or *Porcellio*, whose ova are again several times the size of those of *Asellus*, and yet the differentiation is identical in time with that of *Asellus*.

Part III. — The Later Development of the Germ-layers.

1. *The Later History of the Mesoderm.*

As has been already pointed out, two distinct regions are to be recognized in the Isopod embryo, an anterior one corresponding to the naupliar region and a posterior or meta-naupliar region. In these two regions one finds a very different behavior of the mesoderm. In very young embryos only the naupliar region is represented, the blastoporic region lying immediately behind it, the front edge of the blastopore being formed by a row of ectoderm cells, in some cases at least, eleven in number, while the ectoderm cells in front of this are arranged more or less irregularly, though the orthogonal curves described by Reichenbach ('86) in *Astacus* are more or less plainly visible.

In the earliest stages this ectoderm rests directly upon the yolk, there being no trace of mesoderm or ectoderm below it. As described in preceding pages, the cells of the blastopore area, the mes-endoderm and the vitellophags, immigrate and multiply, forming a plug projecting into the yolk, and later they scatter, principally forward, so that at this stage numerous cells begin to be found beneath the naupliar ectoderm. In none of the forms studied is there to be observed any difference in form or appearance between the mesoderm and the endoderm cells at this stage; in *Jaera* the vitellophags are early distinguishable from the other mes-endoderm cells, but in other forms they do not become distinguishable until the beginning of the scattering of the mes-endoderm plug when

they assume their characteristic function. For convenience, I distinguish here these vitellophag cells from the remaining elements of the mes-endoderm; their significance will be discussed in another section of this part of the paper.

The mes-endoderm cells do not remain irregularly scattered under the naupliar ectoderm very long, but one soon finds them arranging themselves in two bands which diverge as they are traced forward, and extend from what was originally the blastopore region to beneath the ocular lobes, forming what have been termed the mesoderm bands, and lying beneath the regions of the ectoderm in which the cells of that layer are somewhat concentrated, as shown in Fig. 25 for *Jaera* and in Fig. 38 for *Asellus*. In addition in *Jaera* a transverse band extends across from about the anterior end of one mesoderm band to the other, so that occupying the central part of the naupliar region of the embryo there is a triangular area in which there are practically no mesoderm cells (see Fig. 25) and in the anterior portion of which, it may here be stated, the stomodaeal invagination will take place. I have not been able to follow the development of these mesoderm bands in *Porcellio* or *Armadillidium*, but in these as well as in the other forms studied their existence is clearly indicated in later stages when the naupliar limbs begin to form.

When these stages are reached, the mesoderm cells are found to have arranged themselves into groups, which, as the limbs grow out, migrate into their interior, and multiplying there form solid mesodermal axes for them. Other mesodermal cells are scattered beneath the Anlagen of the nervous system and beneath the region where the stomodaeal invagination is preparing, but no special mesodermal groups were found immediately external to the points of origin of the naupliar limbs. At this stage a differentiation of endoderm cells appears owing to the formation of a special group of cells on either side at about the level of the first maxillae, these cells forming the Anlage of the liver lobes. They early arrange themselves in the form of a hollow sphere, which is not complete, however, being open towards the yolk, which the cells proceed to digest. I may say here that I have found

no distinction of a secondary mesoderm from a primitive one at this stage, though later, I believe, cells homologous with Reichenbach's secondary mesoderm are to be found.

The later development of the naupliar mesoderm I have not attempted to follow, and shall turn now to a consideration of what takes place in the meta-naupliar region.

At the time of the scattering of the mes-endoderm cells the ectodermal teloblasts begin to divide in the manner described and grow backwards over the blastoporic region, the entire meta-naupliar ectoderm on the ventral surface being produced by this teloblastic growth. In embryos which are removed from the yolk, it can readily be seen that the meta-naupliar mesoderm, as well as the ectoderm, is produced by a teloblastic growth, though the rhythm of division of the mesodermal teloblasts and their arrangement is very different from that of the ectoblasts. I have succeeded in making out these mesoblasts in *Porcellio*, *Ligia*, and *Cymothoa* with great distinctness, and sections of *Jaera* and *Asellus* show that essentially the same arrangement of them occurs in these forms.

In Fig. 62 is represented a preparation of *Ligia*, which shows the arrangement occurring in that species, and I have chosen *Ligia* for this purpose on account of the discrepancies which my preparations show when compared with the figures given by Nusbaum ('93, Fig. 6). Preparations exactly similar in all essentials have been obtained from *Cymothoa* and *Porcellio*, and also by Bergh from *Mysis*, a fact which strongly suggests that Nusbaum's observations are somewhat incorrect. Lying beneath the ectoblasts, and slightly behind them, are eight mesoblasts arranged in a very definite manner. One is situated on each side of the median line of the embryo; at a slight distance from these on each side are three others, which are separated from one another by intervals shorter than that which separates the innermost one from the cell which lies near the middle line. Immediately in front of each one of these mesoblasts is to be seen a single cell, the products of their last division, and further forward are to be seen three other transverse rows, each consisting also of eight cells, arranged as in the two posterior rows. In the sixth row,

counting from behind forward, the two cells on either side of the median line remain as before, but in the three lateral rows of either side a division of the original cells has occurred, there being on one side six cells arranged in two parallel rows, while on the other side the middle cell has not yet divided, so that only five cells are seen.

In the next row, or since the various transverse rows have evidently a segmental significance, in the next segment (ab'), the multiplication of the mesoderm cells has progressed still further, there being now four cells in a single row representing the original two cells on either side of the median line, while the arrangement indicated for the lateral cells in the segment next behind is complete, and two parallel rows consisting of three cells each are to be found. In the next segment anteriorly (th^1) there are still four median cells, but the lateral rows are again in division, the division apparently affecting the anterior of the two rows already present and leading to the formation of three parallel rows each consisting of three cells. In this segment there is to be seen the first indications of the budding out of the limbs, these structures becoming more pronounced as one passes forward, and at the same time the multiplication of the mesoderm cells progresses rapidly, so that in the preparation at present being described it is impossible to trace any regularity in the division of these cells. In the most anterior of the segments figured, however (th^3), as well as in the two which succeed it five masses of mesoderm cells are to be distinguished: (1) a mass occupying the median line of the segment and unquestionably derived from the two mesoblasts situated on either side of the median line, (2) a mass on either side which corresponds to the limb bud, below which it lies, and (3) a mass, also paired, which lies just lateral to each limb bud.

The regularity of the division of the mesoderm cells is shown more satisfactorily in Fig. 63, which represents the posterior extremity of a very slightly younger embryo of *Cymothoa*. Posteriorly the cells were not perfectly distinct, some of them apparently having remained adherent to the yolk when the embryo was removed from it. In the fourth segment, counting

from behind forwards, one of the median cells is in process of division, and in the fifth segment its division is complete and its fellow is in process of division. In the sixth segment there are four median cells lying all on the same level, an arrangement which indicates a certain amount of migration of the daughter cells, since the division is oblique as is shown in the two segments lying behind. In this sixth segment also two rows of lateral mesoderm cells are found on each side, each row consisting of three cells. The only difference seen from what has been described in *Ligia* is that the median cells begin to divide before the lateral ones, while the reverse seems to be the case in *Ligia*. In the seventh segment the median cells are again in division, each of the four dividing off a cell anteriorly, the eight cells so formed arranging themselves later somewhat irregularly, as may be seen from the eighth segment, which also shows that there are nine cells in each of the lateral masses, arranged in three rows of three cells each and probably formed in the manner indicated in the eighth segment of the figure of *Ligia*.

The regularity of the entire process of growth of the metanaupliar region of the Isopods is most remarkable, and the more one studies it the greater is the wonder it excites. The regular rows of ectoderm and mesoderm cells are wonderful in themselves, and when there is added a more or less definite number of rows for all the species, we see that we are dealing with laws of growth which are at present far beyond our powers of explanation. It is true that the number of ectodermal teloblasts is not always quite constant, though approximately so, but it is exceedingly interesting to find that where, as in *Asellus*, they can be traced back to their earliest differentiation, there is a definite number of them, namely eleven. And this definiteness of number is not confined to the Isopods, but is found also in *Mysis* (Bergh, '93). As regards the mesoblast, however, the number is more constant, eight and eight only occurring in *Cymothoa*, *Ligia*, and *Porcellio;* and again we find exactly the same number in *Mysis*. Nusbaum ('93) in the figure he gives of their arrangement in *Ligia*, as well as in that he gives of *Oniscus*, shows neither the constancy of number nor the regularity of the

earlier divisions which I have found, and the harmony of my observations with those of Patten ('90) on *Cymothoa* and those of Bergh on *Mysis* indicate that his observations on this point have not been conducted as carefully as might be desirable. As is well known, Patten ('90) was the first to describe the mesoblasts and their arrangement in the Isopods, and my results agree essentially with his, except that I have not been able to discover the syncytial connections which he figures as existing in the younger rows. I am not prepared to say that the connecting strands of protoplasm do not exist, indeed, I see no reason why they should not, but in none of the numerous preparations I have made of *Cymothoa* have I been able to discover them.

The relation of the ectodermal and mesodermal transverse rows to the segments of the meta-naupliar region is of some interest, and I have been able to determine that *each row of mesoderm cells is equivalent to a segment.* Thus, in Fig. 62, of *Ligia*, it is clear from the formation of the limbs and the relation of the mesoderm masses to these structures that the mesoderm masses are segmental, and it can also be seen that the masses have been produced by the multiplication of a single transverse row of eight mesoderm cells. In fact the most anterior of the segments represented in the figure is the third thoracic, and the last in which the limb rudiment is visible is the seventh thoracic. Following upon this are seven transverse rows of mesodermal teloblasts which correspond to the six abdominal segments and the telson, the last row of mesoderm cells which, it may be noted, may be regarded as the original mesoblasts, giving rise to the mesoderm of the telson, just as in the annelids the mesoblasts in all probability give rise to the mesoderm of the anal segment.

Does each transverse ectodermal row also represent a segment? This is a question more difficult to answer than when the mesodermal rows were concerned, but I believe that the preparation represented in Fig. 64 indicates what the relation is. This preparation is from a younger embryo of a *Cymothoa* than that represented in Fig. 63, and though it is difficult to be sure whether or not all the mesodermal segments are complete, yet I have reason to believe that they are. This point is

not, however, essential for our present purpose. What is of immediate concern is the relation of the mesodermal and ectodermal transverse rows. The anterior four rows of mesoderm cells are each separated by an interval corresponding to an ectodermal row, and each corresponds to an ectodermal row, while the row immediately in front of the mesoblast corresponds practically to the row of ectoderm cells immediately in front of the ectoblasts. Certain of the ectoblasts are, however, in process of division, and it is clear that this division will result in the interpolation of a row of ectoderm cells between the ectoblasts and the row above which the penultimate row of mesoderm cells lies, so that eventually the same relations of the mesodermal and ectodermal rows as exist more anteriorly will be brought about. The conclusion is, then, that two rows of ectodermal cells primarily correspond to each segment and that therefore *the rhythm of division of the ectoblasts and mesoblasts is not the same, the ectoblasts dividing twice as rapidly as the mesoblasts.* It may be pointed out that while the figure which supports this conclusion is slightly diagrammatic, yet nevertheless the relations of the ectodermal and mesodermal cells were carefully indicated by means of the camera, and the figure therefore represents these relations accurately. It is hardly necessary to state that the occurrence of a greater number of ectodermal rows between each of the mesoderm rows in older segments is due to the division of the cells composing the rows ; the numerical relation just described is to be seen only in segments which have been but recently formed.

Not only is there this definiteness in the number of cells concerned in the formation of each segment, but the number of the segments themselves is definite throughout the entire group of the Isopods. As is well known, there are seven thoracic and six abdominal appendages (not counting the telson) in the Isopods, and if to these be added the segment which bears the maxillipeds and the two maxillary segments, we have in the meta-naupliar region of the Isopods sixteen segments. Since what may be regarded as the primary ectoblasts and mesoblasts are finally located in the telson, it is clear that *during development the mesoblasts must divide ex-*

actly sixteen times and the ectoblasts thirty-two or probably thirty-three times, before they relinquish their teloblastic mode of division.

What determines the cessation of the teloblastic mode of division is a puzzle, but it is accompanied by very decided changes in the appearance of the ectodermal teloblasts. This difference is shown in Figs. 63 and 64. In the latter the ectoblasts are very large and readily distinguishable, while in the former this distinction is entirely lost and they have the same size as the remaining cells of the ectodermal rows. Bergh ('93) has shown that in *Mysis* this is accompanied by a change of position of the spindle in the cell ; so long as the mode of division is teloblastic the equatorial plate lies in front of the middle of the cell, but when it ceases the plate lies at the middle and the cell divides into two equal parts. This stage I have not succeeded in finding, and can only state that a reduction in size of the teloblasts takes place, and that they, together with the mesoblasts and the ectoderm cells lying behind them (the anus making its appearance in the center of these cells), form the telson.

In a transverse section through one of the anterior thoracic segments of a *Cymothoa* embryo (Fig. 65) in which the limbs (*th*) are represented only by ectodermal thickenings, one finds immediately below each of the nerve ganglia (*N*) a mass of mesoderm cells (*cM*) which are evidently the result of the division of two mesoderm cells which were budded off from the mesoblasts which lie on each side of the middle line. Peripherally to these are found some scattered mesoderm cells (*mM*) which represent that portion of the mesoderm which will give rise to the mesodermal tissues of the limb ; in the next sections these are somewhat more abundant, the similarity to Fig. 62 being thus more pronounced than in the section figured. Still more peripherally is to be seen a compact mass of mesoderm (*lM*) which corresponds to the lateral masses seen in the more anterior segments of Fig. 62. In a later stage the arrangement of the mesoderm is very similar. Fig. 66 shows a section through the first thoracic appendage of such a stage, and from it it will be seen that the limbs have be-

come separated by a considerable distance from the nerve ganglia, a separation which increases markedly in the succeeding stages. Beneath the point of origin of each limb is to be seen a portion of the limb mesoderm, and in the transverse section of the maxilliped a core of mesoderm occurs which has evidently wandered into the limb as it grew out from the body. Laterally to the limb mesoderm on each side, is a portion of the lateral mass of the segment, still distinct, and the only marked difference from the section shown in Fig. 65 is the absence of the more median mesoderm masses, which are represented only by a few scattered cells situated on either side of the nervous cord. This is perhaps to be explained by the separation of the limbs from the median line which has already been mentioned, the mesoderm having scattered so as to cover the greater surface now developed.

As to the ultimate fate of the various portions of the mesoderm, it may be stated definitely that the limb mesoderm and the mesoderm of the lateral masses become converted almost entirely into muscle tissue, though the possibility of a certain amount of connective tissue being formed from them cannot be excluded. The fate of the median masses could not be followed, however, though I am inclined to think that they form connective tissue rather than muscle fibers. Consequently I have refrained from applying Bergh's ('93) term of *myoblasts* to the mesodermal teloblasts of the meta-naupliar region, since, though applicable to the three lateral mesoblasts of each side, it is questionable whether it is as properly applied in the case of the two median cells.

In his preliminary paper, Nusbaum ('91) describes with a figure an embryo of *Ligia*, in which the abdominal limbs are not yet formed, and calls attention to the existence of a transitory exopodite in the thoracic appendages. This observation I can confirm, and may add that the exopodites may also be seen in embryos of other Isopods, though less distinctly than in *Ligia*. Nusbaum also calls attention to a patch of cells in each segment situated just laterad of each limb rudiment, describing them as ectodermal thickenings and homologizing them with epipodites. These patches are readily discernible in the

embryos of all the Isopods I have studied, but I must take exception to Nusbaum's interpretation of them. In preparations of entire embryos, such as he figures, they might readily be taken for ectodermal thickenings, but sections show at once that they are really the lateral mesodermal masses shown in my Figs. 65 and 66 (*LM*). An homology of these structures with epipodites seems out of the question ; they do not stand in relation to the limb, but are situated in the pleura when these are developed, and, as stated, give rise to the lateral muscles of the body.

So far nothing has been said regarding the origin of the heart, practically all the teloblastic mesoderm going to form muscle-tissue. Inasmuch as the heart formation concerns the vitellophag cells, a description of its origin will be postponed to the next part of the paper, in which the fate and significance of the vitellophag cells will be discussed.

2. *The Formation of the Digestive Tract and the Later History of the Vitellophags.*

It has been stated in an earlier part of the paper that what are probably to be considered endoderm cells are distinguishable in an early stage of *Jaera* and perhaps also of *Asellus*, but they are recognizable only by their position and not by any histological peculiarities. In the Oniscidæ studied they could not be determined, the entire aggregate of mesodermal cells being identical in appearance. Even in the Asellids they become indistinguishable in the later stages when the scattering of the mes-endoderm takes place, and it has not been possible to trace their later history and their conversion into the liver-lobes, which is, I believe, their fate.

However that may be, the liver lobes first make their appearance as a mass of cells on either side at about the level of the first maxillary segment, and the cells of each lobe early arrange themselves in an epithelium, forming a more or less spherical body which is open towards the yolk, a certain amount of which is enclosed by them and apparently undergoes within them a digestion. At an early stage in *Cymothoa* the spherical

sac becomes drawn out on its posterior surface into three finger-like processes, while in the Oniscidæ and Asellidæ only two processes are developed, and appear at a somewhat later stage of development. These processes give rise to the liver-lobes of the adult, continuing to increase in length with the growth of the embryo. For a considerable time the liver-lobes lie free in the yolk, eventually uniting anteriorly with the posterior end of the stomodaeal invagination, by which the anterior portion of the digestive tract, as far back as the posterior extremity of the stomach, is formed, while the intestine is formed almost completely by the proctodaeal invagination.

In other words I find, as Bobretzky ('74) found in the case of *Oniscus* and as Nusbaum ('93) also finds, that the digestive tract is formed almost altogether from the two ectodermal invaginations, the mesenteron being represented principally by the liver-lobes, only a very small portion of the intestine, just where the liver-lobes unite with it, being possibly endodermal.

The stomodaeal invagination makes its appearance relatively early, and when first clearly distinguishable lies almost on a level with the rudiments of the antennules, if anything a little behind them, slightly later (Figs. 69 and 70 *st*) being distinctly behind them, though in front of the antennae. The invagination presses deeper and deeper into the yolk, and at its posterior extremity enlarges to form the stomach, the liver-lobes, as already stated, uniting with it at its posterior extremity. The proctodaeum appears slightly later than the stomodaeum and when first observable appears as a patch of cells lying a short distance behind the teloblasts (Figs. 62 and 63, *a*) and therefore having the same relation to these structures as had the blastopore in a much earlier stage, though from the mode of growth of the teloblasts backwards over the blastoporic cells, there can be no identity of these latter with the proctodaeal cells. The invagination at first forces its way through the yolk, but tends towards the dorsal surface of it where it lies in the more anterior abdominal segments. It extends far enough forward to unite or almost unite with the stomodaeum, a very small amount of endoderm, as already stated, possibly being interposed between the two invaginations.

Such being the mode of formation of the digestive tract, what is the ultimate fate of the vitellophags, and which of the three germinal layers do they represent?

At the time of the migration of the blastopore cells the vitellophags sink into the yolk, which divides up into masses as has been so frequently described in other Crustacea, there being, however, no formation of secondary yolk pyramids such as occur in *Astacus*. The yolk persists for a long time, even up to hatching in the thoracic region of the body; but it disappears in slightly earlier stages in the abdominal region and here phenomena which accompany its disappearance may be studied. In Fig. 67 is represented a transverse section through the anterior portion of the abdomen of an embryo of *Cymothoa* in which the yolk is beginning to undergo disintegration. Towards the ventral surface on either side is to be seen one of the lateral mesoderm masses whose cells are beginning to be converted into muscle tissue (*mu*), and in their vicinity are to be seen a number of scattered cells, whose origin is I believe indicated on the left side of the figure. Here at the sides above the lateral mesoderm mass are a number of vitellophags still scattered through the yolk, which throughout the whole section has broken up into small and somewhat separated particles, a preliminary to its breaking down into minute granular particles such as are seen in the upper left-hand portion of the figure (*dy*). As this ultimate disintegration occurs the vitellophags are set free and form the scattered cells already alluded to. This setting free of the vitellophags is not confined to the lateral regions of the body, but is to be seen also towards the dorsal surface. Here one finds in the section figured in the middle line the proctodaeum (*pr*) and on either side of it is a row of cells which correspond to Nusbaum's cardioblasts. Their situation in the yolk seems to indicate that they may represent vitellophags, though I have not been able to trace either their origin or their ultimate fate. Above the proctodaeum and on either side are to be found numerous other cells, some of which and probably all are freed vitellophags, and various stages in their aggregation and separation from the yolk may be found by tracing the series of sections forwards.

Some of these cells are becoming muscle cells (*mu*) and will eventually produce the longitudinal dorsal muscles of the abdominal region, while others do not become thus transformed, but retain their original character in much later stages. Whether or not they become blood corpuscles I cannot certainly state, though from what I have seen in sections through later stages I am inclined to believe that that is, in part at all events, their fate.

In the oldest embryos of *Cymothoa* which I possess the organs are all fully formed, and there is no trace of yolk in its original condition to be found. In slightly earlier stages the digestive tract is complete, and there is still a certain amount of yolk present in the thoracic region of the body, and within it vitellophags are to be seen, and there does not seem to be any possibility that these cells enter into the formation of the digestive tract; in fact I have seen no indications that any of the vitellophags do so. In the latest embryos (Fig. 68) the spaces between the various organs are occupied by a granular mass (*dy*) which resembles in appearance the granular disintegrated yolk seen in the dorsal region of Fig. 67 (*dy*), and I believe this matter is of the same nature and represents the final condition of the yolk. It occupies the place of the blood plasma, and has imbedded in it a certain number of amoeboid cells (*vi*), the blood corpuscles, which are, there is every reason to believe, persistent vitellophags. This result agrees perfectly with the observations of Nusbaum on *Mysis* ('87), and I can confirm for *Cymothoa* the statements contained in the last two paragraphs of page 192 of his paper.

Not all the vitellophag cells take part, however, in the formation of persistent tissues. Some appear to break down, being enclosed within the liver lobes, and others seem to disintegrate independently of the action of the liver, giving rise to particles of chromatin such as may be seen in Fig. 67 (*Cr*) scattered among the cells at the surface of the yolk, and resembling the chromatin nebulae, which have been described by various students of Decapod embryology. So far as I have seen, however, such disintegrating cells are few in the Isopods, and are to be found only in the latest stages of embryonic development.

My belief, then, with regard to the vitellophags, is that they take no part in the formation of the digestive tract, that some of them disintegrate and disappear, but that the majority take part in the formation of persistent structures, such as connective tissue, muscle tissue, blood corpuscles, and perhaps even of the heart itself. These are what are generally recognized as mesodermal structures, and I do not believe that the vitellophags should be regarded as being anything but mesoderm.

3. *General Considerations on the Formation of the Germ-layers in the Crustacea.*

I wish, in the first place, to emphasize once more the distinction existing between the mode of formation of the naupliar and meta-naupliar regions of the Isopods, a distinction already pointed out by Bergh ('93) as obtaining in *Mysis*. The latter portion, so far as its ectoderm and the greater bulk of its mesoderm are concerned, is produced by a teloblastic growth, while in the naupliar region no such general method of growth is pronounced. If the egg-embryo proper be regarded as consisting of the blastopore region and the portion of the embryo immediately in front of this, then the meta-naupliar region may be regarded as a portion of the body normally developed after hatching, but in the Isopods developed, in accordance, probably, with the occurrence of a brood-pouch, before the embryo begins to lead a free life. In other words, the development of the Isopods points back to a period when a free-swimming *Nauplius* occurred in the development of the ancestors of the group, the egg-embryo being a *Nauplius*, if one may so express it, just as it is in the *Peneus*, for example, in which the post-mandibular segments develop only after hatching. In the Annelida a teloblastic mode of growth has been described, more especially in connection with the mesoderm, and if the development of such a form as *Polygordius* be taken as a type of the larval method of development in the Annelids, an interesting comparison may be made. Thus in *Polygordius* the Trochophore may be regarded as the egg-embryo proper, the addition of new segments being associated with a teloblastic growth of the mesoderm,

and consequently the same relations obtain between the trochophoral and meta-trochophoral regions of *Polygordius* as have been noted as obtaining between the naupliar and meta-naupliar regions of the Crustacea as represented by *Mysis* and the Isopoda.

Whether these similar relations indicate an homology of the two regions in the two groups or not I am not prepared to state. If the teloblastic growth is homologous in the two groups, that is to say, if it has been derived from a common ancestor, then it follows that the trochophore and the *Nauplius* are homologous, but, on the other hand, there is no evidence that this is the case; indeed, the marked dissimilarity in the details of the arrangement of the teloblasts points strongly against any such assumption, and suggests that the teloblastic mode of growth has been developed independently in the two groups, and is to be regarded merely as a provision for rapid growth. The fact that even certain of the organs, such as the nerve ganglia, also show teloblastic growth in the Crustacea and Insects (Wheeler, '91), lends support to this view of the question.

I have indicated that my results as to the formation of the germ-layers agree essentially with those of Bergh on *Mysis*. In one important particular, however, there is a difference in our interpretations of the phenomena. As I understand Bergh's statements, the entire mass of blastoporic cells becomes converted into vitellophags, endoderm, and eight mesoblasts, and the conclusion is that the mesoderm of the naupliar region is produced by the mesodermal teloblasts. This is certainly not the case in the Isopods, in which, as I have stated, a considerable number of the blastoporic cells give rise to mesoderm cells, independent of the mesodermal vitellophags, and teloblastic growth is found in connection with neither the mesoderm nor the ectoderm in the naupliar region of the body. We may consider the naupliar region to be the embryo proper, and the blastopore being an embryonic structure gives rise to the embryonic mesodermal and endodermal tissues, making provision, however, by the formation of the mesodermal teloblasts for the rapid growth of the later larval mesoderm.

So many accounts of the formation of the germ-layers in the Crustacea have been published, and the various accounts have been so frequently brought together in *résumé*, that I may be pardoned for refraining from entering into a lengthy, critical review of the works of my predecessors in this line. It may be pointed out, however, that the various accounts may be divided into two groups: (1) Those which derive all the mesodermal and endodermal cells primarily from the blastoporic cells, and (2) those which assign a portion at least of the mesendodermal formation to extra-blastoporic regions. My results upon the Isopods belong to the first group, and are in harmony, in this respect, with those of the authors, such as Grobben ('79 and '81), Samassa ('93), and Brauer ('92), who have studied the formation of the germ-layers in the lower Crustacea, as well as with those of Bobretzky ('74) on *Oniscus*, of Bergh ('93) on *Mysis*, of Brooks ('83) on *Lucifer*, and of Reichenbach ('86) on *Astacus*, not to mention other students of the Decapods. In the last forms, however, several authors, as for example, Kingsley ('87), Ishikawa ('85), have described the occurrence of cells imbedded in the yolk before the differentiation of the blastoporic cells, a condition probably due to the migration or delamination of some of the blastula cells at an early stage, as has been described by Herrick ('92). This is a phenomenon apparently peculiar to the Decapods, and I do not propose to discuss its significance here; with the exception of this peculiarity the mes-endoderm formation of the Decapods is localized in the so-called blastoporic region.

To the second group belong the observations of several authors whose results require more detailed notice. Nusbaum has described in *Mysis* ('87) and *Ligia* ('91) the formation of mesoderm from the ectodermal cells along the entire length of each of the lateral bands of the naupliar region of the embryo, and Lebedinski ('90) holds essentially the same view as to the origin of the mesoderm in *Eriphya*. As regards *Mysis* Bergh has given a very positive statement as to the incorrectness of Nusbaum's observations on this point, and as already stated there seems to me to be no evidence in the Isopod that such a mode of formation of the mesoderm obtains. Nusbaum evi-

dently has not carried his observations far enough back to observe the scattering forward of the mesoderm from the blastopore, and finding mesoderm cells below the lateral ventral bands has imagined that they have split off from them. It remains to be seen whether further observations on the Brachyura will confirm Lebedinski's statements; it may be noted, however, that the recent work of Cano ('93) on *Maja* does not afford any support to them.

In this connection mention may be made of Weldon's ('92) observations on *Crangon*, in which he finds a patch of mesoderm cells on either side of what he takes to be the blastopore and beneath apparently the posterior ends of the lateral embryonic bands. I have observed the same arrangement in *Palaemonetes* and *Virbius* but cannot agree with the interpretation Weldon puts upon it. He regards what he has found as a partial confirmation of Nusbaum's views, but in reality the conditions are quite different, since Nusbaum derives the mesoderm from the ectoderm of the ventral surface of the naupliar region, while, even granting that Weldon's views are correct, it is formed from the meta-naupliar region in *Crangon*, in my opinion a very important difference. But, in addition, Weldon identifies what he terms the ventral neuro-muscular plates of his Fig. 6 with the thoracico-abdominal plates of *Astacus*, and this is where I believe he has fallen into error. As I interpret the similar appearance in *Virbius* these neuro-muscular plates are in reality part of the blastopore which is elongated laterally, and they are entirely composed of mesoderm cells, not yet being covered in by ectoderm, a view which, it seems to me, is corroborated by the appearance which is seen in section and which Weldon represents in his Fig. 16. His Fig. 6 is not at all comparable, as he supposes, to Reichenbach's Fig. 3, but is of a much earlier stage. Why the mesoderm should arise as two lateral masses rather than as a single mass situated immediately in front of the vitellophag-endoderm mass, may perhaps be explained by the separation of the two lateral ventral bands in early stages.

The remaining observations which belong to the second group of results require but little discussion. The views of

Reinhard ('87) and Roule ('91, '92, '92 *a*) have already been stated (p. 96), and it is very clear that they receive not the slightest confirmation from the forms I have studied, and are at variance with the results of the other observers who have studied the embryology of the Isopods. As regards the Amphipods much has yet to be done before a proper idea of their early development is obtained; the results of Dr. Sophie Pereyaslawzew ('88) and her co-workers Mesdames Rossiiskaya – Koschewnikowa and Wagner, are evidently quite inadequate, the mesoderm being stated to arise in connection with the limbs, evidently not having been traced even approximately to its origin. The results of Della Valle ('93) though much more carefully worked out, still leave much for later investigation, it being impossible to harmonize them with what occurs in other Crustacea.

There are reasons then for doubting the correctness of the views of those authors who ascribe an extra-blastoporic origin for the mesendodermal elements in the Crustacea except in the case of the Decapods, and since these are the most highly specialized of all the Crustacea, the precocious formation of vitellophags which they show may with justice be regarded as a secondary phenomenon. It remains to consider what is to be regarded as the blastopore in the Crustacea, and what the significance of the various phenomena which have been described in connection with it.

One naturally turns to the simpler forms to get an idea as to the primitive character of the blastopore, and in the Phyllopods one is at once met by two striking facts, (1) the mesoderm and endoderm have a common origin and cannot at the time of their formation be distinguished from one another, and (2) they arise by the immigration of certain cells into what would be the blastocoel were the yolk not present, there being no indication of an invagination. These two facts are true of *Daphnia* and *Daphnella* (Samassa, '93) and of *Branchipus* (Brauer, '92), and according to Samassa's account of *Moina* also, though Grobben ('79) describes for this form an invagination, and also an early differentiation not only of the mesoderm from the endoderm, but also of two distinct portions of the mesoderm.

These results, however, since Samassa's observations, stand in need of confirmation before they can be accepted. In *Cetochilus* Grobben ('81) describes an invagination of the endoderm and a distinct differentiation of the mesoderm from this, but in description after description of Crustacean development one reads of a solid endodermal plug, a condition which seems to be of the most frequent occurrence.

However, not unfrequently it is possible to distinguish a differentiation of the blastoporic cells, if the term blastopore can be applied here, and in this respect the phenomena described in preceding pages are exceedingly interesting. In *Cymothoa*, *Ligia*, and the other Oniscidæ, and practically in *Asellus*, no differentiation can be made out, but in *Jaera* a well-marked differentiation of a portion of the mesoderm, namely of the vitellophags, occurs. The differentiation of the endoderm can be disregarded, since it is one of position only and does not persist, so that we have in that form an anterior mesendodermal mass and a posterior mesodermal group of vitellophags. Such a differentiation is peculiar; it is not a differentiation of endoderm and mesoderm, but a specialization of a certain part of the mesoderm from the remainder, and it is interesting as indicating a process of precocious segregation which may be carried to extreme lengths, and to which the mesoderm, on account of the multiplicity of organs which arise from it, is especially susceptible. Thus the remarkable differentiations of teloblast cells which are found in such cases as *Lumbricus* (Wilson, '89) and *Clepsine* (Whitman, '78) are to be regarded as cases of this kind. From the general ectoderm are differentiated two teloblasts which give rise to the ventral nerve cord; and from the general mesoderm are differentiated the nephroblasts. We find, too, the interesting peculiarity that the nephroblasts pass into the blastocoel at a later period than do the mesoblasts, and the conclusion has been drawn that the nephridia therefore are of ectodermal origin in these cases. Is there any necessity for such a conclusion? It seems to me that the phenomenon is simply the culmination of the process of differentiation of portions of the germ-layer, the segregation of the nephroblasts from the mesoblasts having proceeded so

far that they act independently of them. In fact we see this same phenomenon in various degrees of perfection in the differentiation of the mesoderm from the endoderm, for phylogenetically the mesoderm is derived from the endoderm, as may be seen for instance in the group of the Turbellaria and as is indicated in the ontogeny of many forms. In some cases, *e.g.*, the Echinoderms, the two layers separate perfectly only after they have passed into the blastocoel; in other cases they are differentiated from one another in the blastula and pass inward together, while in other cases again the migration or invagination of the endoderm may precede that of the mesoderm, or the mesoderm, either in whole or in part, may pass inward before the endoderm. To speak of the origin of the mesoderm in one case from the ectoderm and in another from the endoderm shows a want of appreciation of the phylogenetic significance of the mesoderm and of the possibilities which result from differentiation.

Primarily, then, in the Crustacea there was the formation of a blastula whose cavity was more or less completely filled with food yolk, and there was a differentiation of mesendodermal cells by immigration, the distinction of mesoderm from endoderm only supervening later. I do not mean to assert that the more remote ancestors of the Crustacea may not have shown an invagination of the epibolic or even of the embolic type; in reality the distinction between invagination and immigration is so slight that one may readily become converted into the other, and the only question of interest in connection with the two phenomena is as to which is the most primitive phylogenetically. I see no reason to withdraw from the position I have taken on this question in earlier papers, in which I announced myself as a supporter of Metschnikoff, and do not intend to discuss the question here. I believe, firmly, however, that the cases of invagination to be found within the Crustacea are secondary.

If these cases be examined, some interesting relations are to be found, relations which involve a correct appreciation of the significance of the vitellophag cells. I have already stated my opinion that these structures are to be regarded as mesoderm

cells, and may point out that this view is in harmony with those of Samassa ('93) and Nusbaum ('87). Most authors who have studied the development of the Decapods have considered the vitellophags to be endoderm, though Herrick ('92) comes to the conclusion that some of them at least give rise to mesodermal structures. When we come to examine critically the reasons for the belief that they are endodermal, one finds that they do not rest on direct observation, but rather upon analogy with what is supposed to take place in *Astacus*. Here we have an invagination lying behind the region from which the ordinary mesoderm arises, and this is represented in the majority of other Decapods by a plug of cells which give rise to the vitellophags, and may in certain cases show indications of a cavity, as in *Crangon* for instance (Weldon, '92). If, then, the invagination of *Astacus* gives rise to the mesenteron, as it is supposed to do, what more natural than to suppose that the vitellophags assist in the formation of the endoderm in other forms? I know of no case, however, in which they have been actually traced to such a final destination, and the whole question comes down to the significance of the invagination of *Astacus*, granting the homology of the vitellophags with the cells which form the secondary yolk-pyramids of that form. Let us examine, then, the relations in *Astacus*, as shown in Reichenbach's beautiful monograph ('86).

It seems to me that we have to distinguish, according to Reichenbach's descriptions, two sets of elements in the invagination, namely, the cells which absorb the yolk and form the secondary yolk-pyramids, and which, for exact comparison, we may here call the vitellophag cells, and certain cells which do not absorb the yolk, and which form what Reichenbach terms the entoderm plate. From the entoderm plate the liver-lobes arise, and it takes part, also, in the formation of a part of the mesenteron. The important question is how much of the mesenteron is derived from it, and what evidence is there that the yolk-pyramids contribute to the formation of the mesenteron. In the oldest stage which Reichenbach figures a considerable amount of yolk is still present. The stomodaeal and proctodaeal invaginations are well developed, the latter being in

contact with a posterior prolongation of the mesenteron, which Reichenbach describes as being formed from the entoderm-plate cells. The ventral wall of the mesenteron is formed of similar cells, as is also a certain amount of the dorsal wall, and it would require but a comparatively slight extension of the yolkless cells to complete the mesenteron and shut off from it completely the yolk-pyramids.

Unfortunately, observations on the later stages of *Astacus* are wanting to render such an interpretation of the formation of the mesenteron a certainty, but I hold that it is one which is more in harmony with the cases in which the fate of the vitellophags has been fully traced up to the disappearance of the yolk, than the view which is generally held. And, furthermore, it harmonizes with certain observations of Reichenbach himself on the yolk-pyramids. It is easily recognizable that as development proceeds in *Astacus* there is a marked diminution of the number of secondary yolk-pyramids, and there is good reason to suppose that this is associated with the formation of the so-called secondary mesoderm cells; indeed, Reichenbach has traced the formation of these cells to the yolk-pyramids. In part, then, the yolk-pyramids give rise to mesoderm elements, and their entire conversion into such elements does not seem to me at all improbable.

If these views be correct, then we have in *Astacus*, and probably in *Eupagurus* as well (Mayer, '77), an invagination which includes both endodermal and mesodermal elements, and there is a further formation of mesoderm by immigration immediately in front of the invagination. This condition is derived from one in which no early differentiation of endoderm, mesoderm, and vitellophags can be recognized, and from this same condition is to be derived the arrangement seen in *Jaera*, where the ordinary mesoderm and endoderm form a common undifferentiated mass while the vitellophags are sharply marked off, as well as the arrangement described for *Lucifer* by Brooks ('83), in which there is an invagination of endoderm and the differentiation of two cells which migrate into the blastocoel before invagination, and apparently represent both the mesoderm and vitellophags.

Whether or not these views are correct later observations will determine, but they permit of a reduction of the developmental phenomena of the Crustacea to a common type, and indicate how the various modifications described have been brought about.

Part IV. — Notes on the Development of Certain Organs.

The observations recorded in this portion of the paper are fragmentary, and consist simply of a few facts which have been incidentally noticed concerning the development of certain organs, and which do not fall strictly within the limits of this paper as originally laid out.

The development of the nervous system I hope some time in the future to work out thoroughly, and shall merely notice here two points concerning it. The first of these is the occurrence of a teloblastic mode of growth of the nerve ganglia in the Isopods, of the same character as that described by Bergh ('93) as occurring in *Mysis*, and similar to what Wheeler ('91) has observed in insect embryos.

The second point is the occurrence in the embryos of all the Isopods I have studied of a pair of ganglia, unaccompanied by corresponding limbs, and lying in front of the antennular ganglia. These are shown in Figs. 69 and 70, of which Fig. 69 represents an embryo of *Cymothoa*, and Fig. 70 one of *Jaera* in a slightly later stage of development. From these figures the following arrangement of the ganglia can be seen. Anteriorly on either side are the large optic ganglia (*op*), and nearer the median line two elongate oval masses, which are to be regarded as the cerebral ganglia proper (*cc*), the optic ganglia being phylogenetically probably a secondary differentiation from these. Behind and externally to the cerebral ganglia is to be seen on either side a thickened mass of tissue (*G*), which is the ganglion to which special attention is directed here, and behind it lies the antennular ganglion (*aG*), situated in front of the mouth invagination (*st*), the other two naupliar ganglia (*at G* and *mG*) lying behind this structure.

In later stages the ganglion *G* as well as the antennular ganglion, and later still the antennary, fuse with one another and with the cerebral ganglion to form the syncerebrum of the adult. The occurrence of this supernumerary ganglion, whose presence indicates the existence of a segment between the eye-bearing and the antennular segments, has already been described by Bumpus ('91) as occurring in the Lobster, and in that form what may possibly be transient indications of a pair of appendages corresponding to the ganglia are developed. I have not been able to detect in the Isopods any indication of appendages, but there can be no doubt that the ganglia I have described are homologous with those seen by Bumpus. From the abstract of Nusbaum's paper ('93) it seems that he has observed what he takes to be a pair of praeantennular ganglia in *Ligia*, which are not, however, identical with those I have found, having an entirely different position and being formed by a splitting off of a portion of the antennular ganglia. The praeantennular ganglia of *Homarus* and those I have described here arise quite independently of both the cerebral and the antennular ganglia, and have, I believe, a segmental value. I have seen thickenings in the region in which they are indicated by Nusbaum, but what their significance may be I am not prepared to state.

I do not intend to follow out the questions of homology which the presence of a praeantennular segment suggests, especially as they have already been discussed by Kingsley ('94). It seems to me that further observations, especially upon the developments of the Entomostracan forms, are necessary before any certain conclusions can be formulated.

In front of the mouth in Fig. 70 two elevations (*ep*) are seen, which later stages show uniting to form the upper lip, and behind the mouth on a level with the mandibles two other elevations (*Mt*) are developed, which give rise eventually to the metastoma. There has been a certain amount of discussion as to whether the metastomal elevations are to be regarded as of metameric value and as having the same significance as limbs. The evidence furnished by the Isopods points to a negative answer to this question, since no nerve-ganglion or neuromere which can be assigned to them is distinguishable,

and furthermore it is to be noted that they do not appear contemporaneously with the limbs, making their appearance long after the limbs are readily distinguishable.

Finally I have introduced two figures (Figs. 71, 72) representing late stages in the development of *Jaera*, which illustrate a point of some significance in connection with the phylogeny of the Isopods. In the adults of these forms there is no indication of the presence of a carapace, but in Fig. 71 a fold (*car*) is clearly seen which extends backward to behind the first pereiopod (th^2) and represents a rudimentary carapace. In the stage represented in Fig. 72 this fold covers relatively a smaller extent of the body, its posterior margin being on a level with the interval between the maxilliped and the first pereiopod. I think there can be no question but that this fold represents a rudimentary carapace, and points to the derivation of the Isopods from ancestors possessing a more or less perfectly developed carapace fold. Whether these ancestors are most accurately represented to-day by the Schizopods, as some have maintained, or by the Cumacea remains to be seen, contributions on the development of the latter group being much required, the observations of Blanc ('85) leaving many points in connection with their development unsettled.

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Woods Holl, Mass.,
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EXPLANATION OF FIGURES.

Lettering used uniformly throughout the figures.

a = anus.
ab = abdominal limb.
aG = antennular ganglion.
At^1 = antennule.
At^2 = antenna.
AtG = antennary ganglion.
B = dorsal blood vessel.
Car = carapace.
ce = cerebral ganglion.
cM = central mesoderm mass.
cp = central protoplasm.
cT = central ectodermal teloblast.
D.O. = dorsal organ.
dy = disintegrated yolk.
E = eye.
Ec = ectoderm.
Ep = upper lip.
G = praeantennular ganglion.
h = heart.
L = liver.
l = liver lobe.
l.en = liver endoderm.
lM = lateral mesoderm mass.
L.O. = lateral organ of *Asellus*.
l.v.b. = lateral ventral band of embryo.
me = mesoderm.
MEn = mes-endoderm.
meT = mesodermal teloblast.
mG = mandibular ganglion.
mM = limb mesoderm.
Mn = mandible.
ms = Mittelstrang.
mt = metastoma.
mu = muscle.
Mx^1 = first maxilla.
Mx^2 = second maxilla.
Mxp = maxilliped.
N = nerve ganglion.
op = optic ganglion.
pn = protoplasmic network.
pp = peripheral protoplasm.
pr = proctodaeum.
st = stomodaeum.
T = ectodermal teloblast.
tb = transverse band of embryo.
te = telson.
th = thoracic limb.
tr = teloblastic row.
y = yolk.

EXPLANATION OF PLATE V.

All the figures on this plate are of *Jaera marina.*

FIG. 1. Transverse section of adult ♀. *Ov* = ovary; *Ger* = germinal region of Ovary; *Sp* = chitinous portion of Spermatophore; *D* = intestine; *DC* = digestive cœcum; *N* = ventral nerve cord; *B* = dorsal blood vessel.

FIG. 2. Section of immature ovum showing protoplasmic network. For the sake of clearness the yolk granules have been omitted. *fc* = follicle cells.

FIG. 3. Section of mature ovum showing the peripheral and central protoplasm, the latter containing the nucleus. The network not visible.

FIG. 4. Ovum just after the expulsion of the polar globules (*pg*) drawn from living specimen. The dark structure in center is the segmentation nucleus with its surrounding protoplasm seen indistinctly through the yolk. *ch* = chorion; *ym* = yolk membrane.

FIG. 5. Optical section of ovum in the two-celled stage.

FIGS. 6 and 7. Optical sections of ova in the four-celled stage showing different stages in the rotation of the cells.

FIG. 8. Ovum during the formation of the eight-celled stage. The figure is from a cleared preparation so that all the nuclei are visible.

FIG. 9. The eight-celled stage completed, from a cleared preparation.

FIG. 10. Section of ovum in the eight-celled stage.

FIG. 11. Surface view of posterior pole of ovum in the 16-celled stage. The two terminal endodermal cells are surrounded by a circle of *seven* cells.

FIG. 12. Surface view of posterior pole of the 16-celled stage in which the number of cells in the circle has been reduced to *six*.

FIG. 13. Surface view of the 32-celled stage seen from ventral surface.

FIG. 14. Surface view of the 64-celled stage seen from the side.

FIG. 15. Surface view of posterior extremity of ovum passing into the succeeding stage.

FIG. 16. Surface view of ovum in the succeeding stage seen from the side.

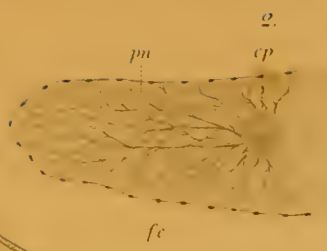
FIG. 17. Ventral view of ovum in which the concentration of the cells towards the ventral surface is taking place.

FIG. 18. Dorsal view of the same ovum.

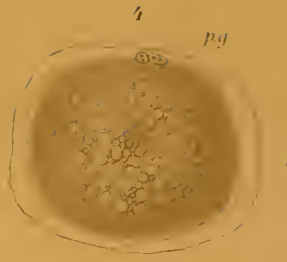
FIG. 19. View of ovum in which the concentration is complete.

FIG. 20. Longitudinal section of ovum in the 32-celled stage.


1
Sp
Ect
Ger
V
I
Dc
X


2.
pm
cp
fc


3
pp
cp
y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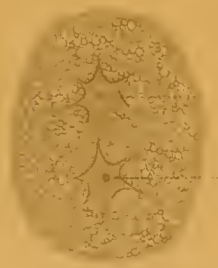
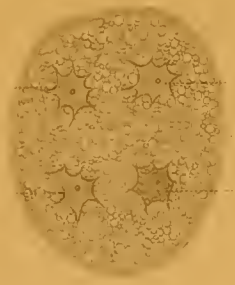
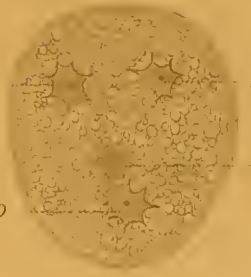
pg
ym
en

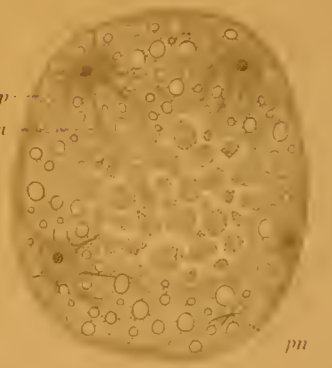

5
pp
A
C


6.
A
B
pp
D
C

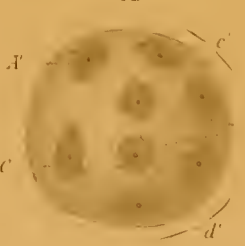

7
A
B
a'
pp
C
c'
D


8.
b'
a'
C
d'
c'
D


9.
b'
a'
B
A
d
C
c'
D


10.
pp
pm


11.
A
C
c'
vi
c''
d'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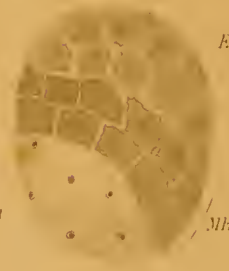
A
c'
C
vi
d'


13.
Ec
MEn
vi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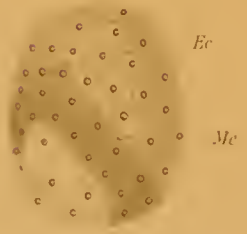
Ec
vi
MEn


15.
Mc
vi
Len


16.
Ec
Len
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17.
Ec
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18
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Mc
vi


19
Ec
Mc
vi
Len


20.
Ec
MEn
Mbr
vi

EXPLANATION OF PLATE VI.

FIG. 21. Sagittal section through an egg of *Jaera* showing the mesoderm plug (*me*) and the teloblastic cell of the ectoderm plate (*T*).

FIG. 22. Sagittal section through egg of *Jaera* showing the beginning immigration of the endoderm cells (*en*) to form the vitellophags. The cells of the mesoderm plug have begun to scatter and the teloblastic growth of the ectoderm has produced short rows of cells (*tr*).

FIG. 23. Ventral view of embryo of *Jaera* in which the teloblastic growth has carried the ectoderm tract over the mesoderm plug.

FIG. 24. Side view of the same embryo showing the patch of optic cells (*E*) and the rows of cells extending to it from the ectoderm plate.

FIG. 25. View of anterior half of an embryo of *Jaera* in which the yolk is about two-thirds overgrown, showing the scattered arrangement of the cells in the naupliar region and the teloblastic rows in the meta-naupliar.

FIG. 26. View of posterior half of the same embryo showing the row of teloblasts (*T*) the central one of which (*cT*) is very well marked.

FIG. 27. Portion of posterior half of an older embryo somewhat broken by pressure showing the row of teloblasts (*T*) and the "Mittelstrang" (*ms*). The dark transverse bands (*me*) in the anterior part of the figure represent mesoderm masses showing through the superjacent ectoderm.

FIG. 28. Egg of *Asellus Communis* in the two-celled stage viewed as a transparent object.

FIG. 29. Egg of *Asellus* in four-celled stage.

FIG. 30. Egg of *Asellus* in eight-celled stage.

FIG. 31. Egg of *Asellus* passing into the 16-celled stage.

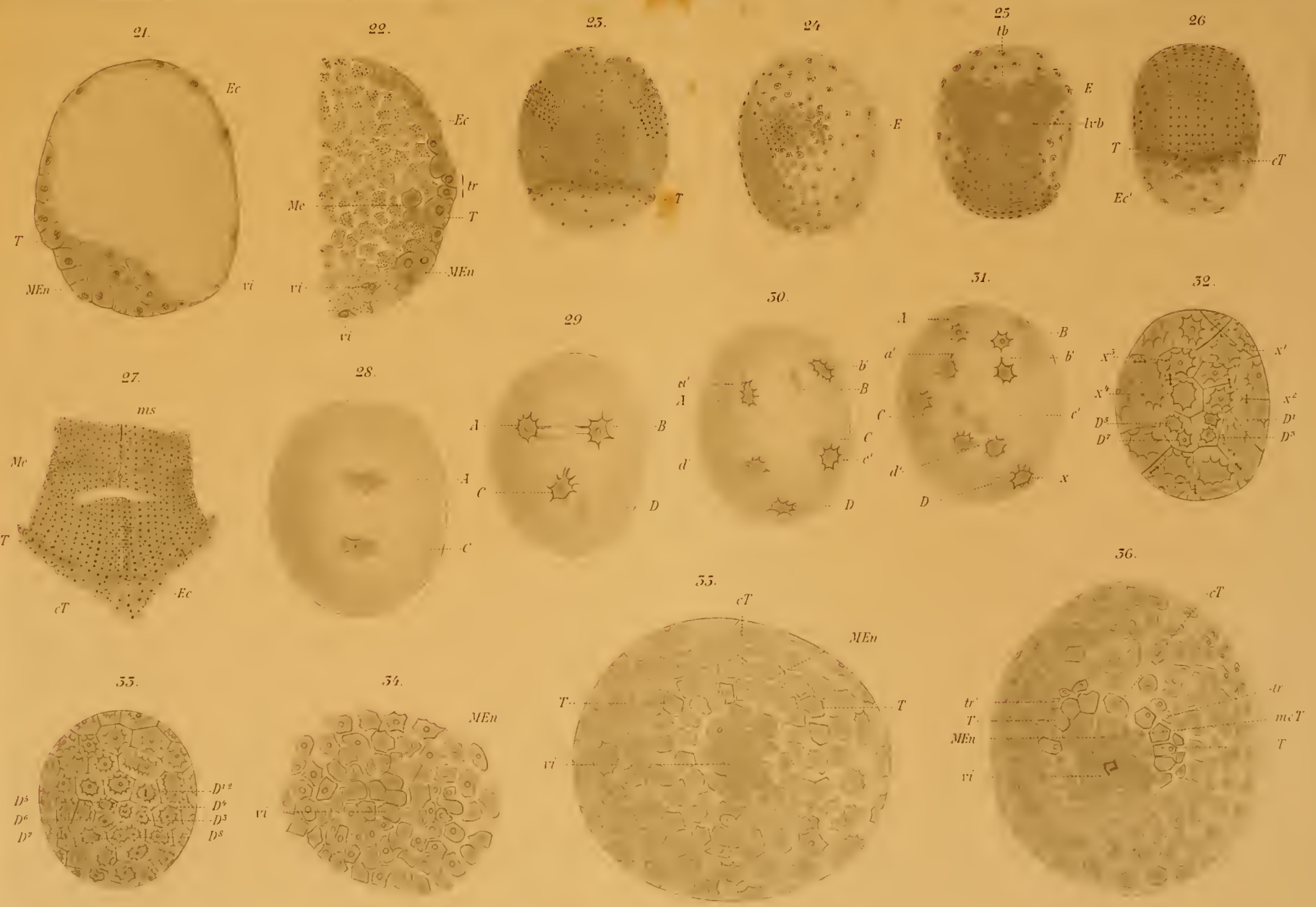
FIG. 32. Egg of *Asellus* in the 64-celled stage viewed as an opaque object.

FIG. 33. Egg of *Asellus* in the 128-celled stage.

FIG. 34. Blastoderm of *Asellus* represented as if removed from the ovum.

FIG. 35. Egg of *Asellus* at the time of the differentiation of the eleven primary ectodermal teloblasts.

FIG. 36. Egg of *Asellus* just after the first division of the ectodermal teloblasts.


21.
Ec
T
MEn
vi
22.
Ec
tr
Mc
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23.
T
24
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lvb
26
T
cT
Ec'
27.
ms
Mc
T
cT
Ec
28.
A
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C
29
A
B
C
D
30.
a'
b'
A
B
C
c'
d'
D
31.
A
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a'
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C
c'
d'
x
D
32.
x'
x²
x⁴
D⁵
D⁷
D¹
D³
33.
D⁵
D⁶
D⁷
D¹·²
D⁴
D³
D⁸
34.
MEn
vi
35.
cT
MEn
T
T
vi
36.
cT
tr
tr'
T
mcT
MEn
T
vi

EXPLANATION OF PLATE VII.

FIG. 37. Embryo of *Asellus* represented as spread out flat.

FIG. 38. Embryo of *Asellus* in stage later than Fig. 37 showing the teloblastic growth of the meta-naupliar portion.

FIG. 39. Ovum of *Porcellio* in the one-celled stage. The thickness of the peripheral protoplasm (*pp*) in this and the next figure is slightly exaggerated.

Fig. 40. Ovum of *Armadillidium* in the two-celled stage.

FIG. 41. Four-celled ovum of *Armadillidium*.

FIG. 42. Section through a four-celled ovum of *Porcellio*.

FIG. 43. Eight-celled stage of *Armadillidium*.

FIG. 44. 16-celled stage of *Porcellio*. The lines joining the various cells indicate their origin from the eight-celled stage.

FIG. 45. 16-celled stage of *Armadillidium*, viewed from the posterior pole.

FIG. 46. 32-celled stage of *Armadillidium*, viewed from the posterior pole.

FIG. 47. Section through an ovum of *Porcellio* in the 32-celled stage.

FIG. 48. Ovum of *Porcellio* in the 64-celled stage.

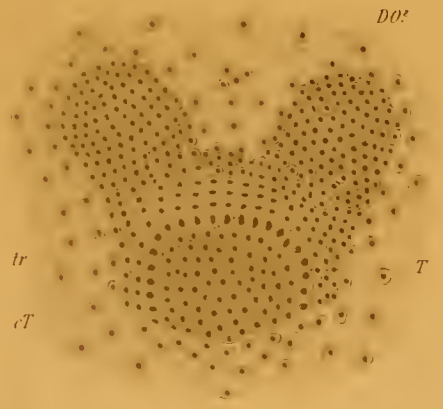
FIG. 49. Ovum of *Porcellio* in the succeeding stage when the concentration of the blastoderm around the mes-endoderm (*m.en*) is beginning.

FIG. 50. Ovum of *Armadillidium* in a stage slightly later than that of Fig. 49.

FIGS. 51 and 52. Still later stages of *Armadillidium*.

FIG. 53. Blastoderm of *Porcellio* in situ.

37



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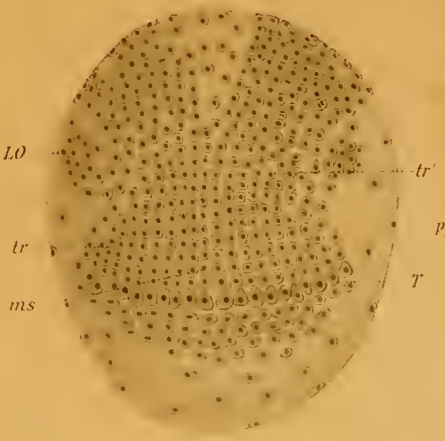

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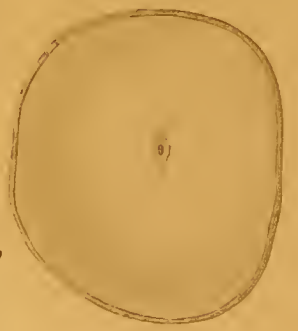

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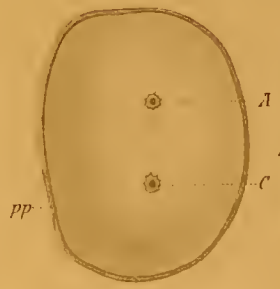

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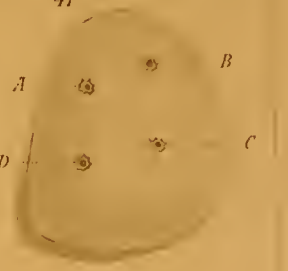

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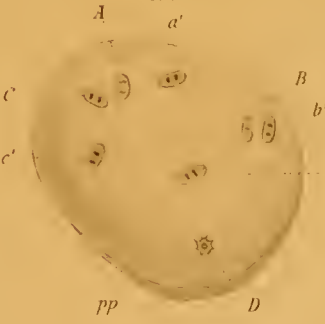

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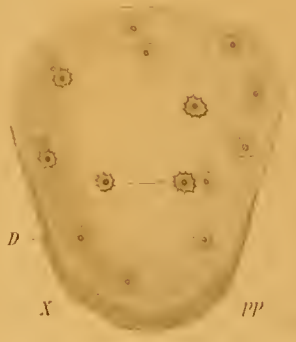

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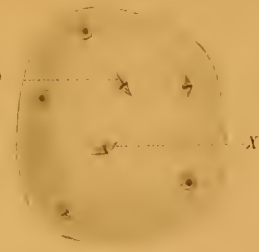

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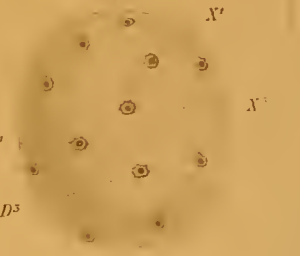

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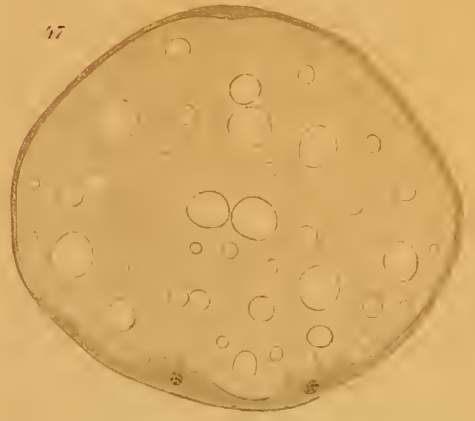

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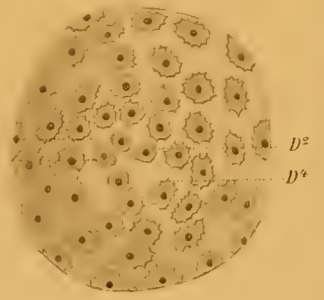

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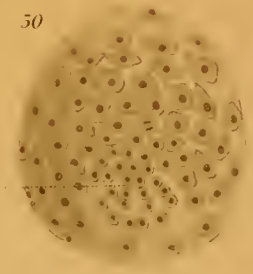

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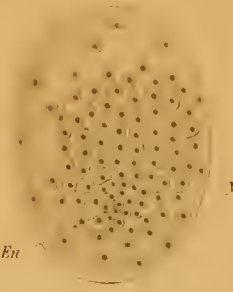

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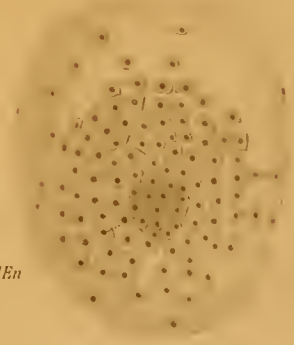


EXPLANATION OF PLATE VIII.

FIG. 54. Blastoderm of *Porcellio* at stage when the mes-endoderm cells are beginning to sink beneath the surface.

FIG. 55. Section through a blastoderm of *Porcellio* of the same age as that of the preceding figure, showing the lower layer cells in the blastoporic region.

FIG. 56. Blastoderm of *Porcellio* at stage just before the scattering of the mes-endoderm cells.

FIG. 57. Blastoderm of *Armadillidium* at the time of differentiation of the ectodermal teloblasts.

FIG. 58. Blastoderm of *Armadillidium* with the ectodermal teloblasts more perfectly differentiated.

FIG. 59. Embryo of *Armadillidium*, commencement of teloblastic growth.

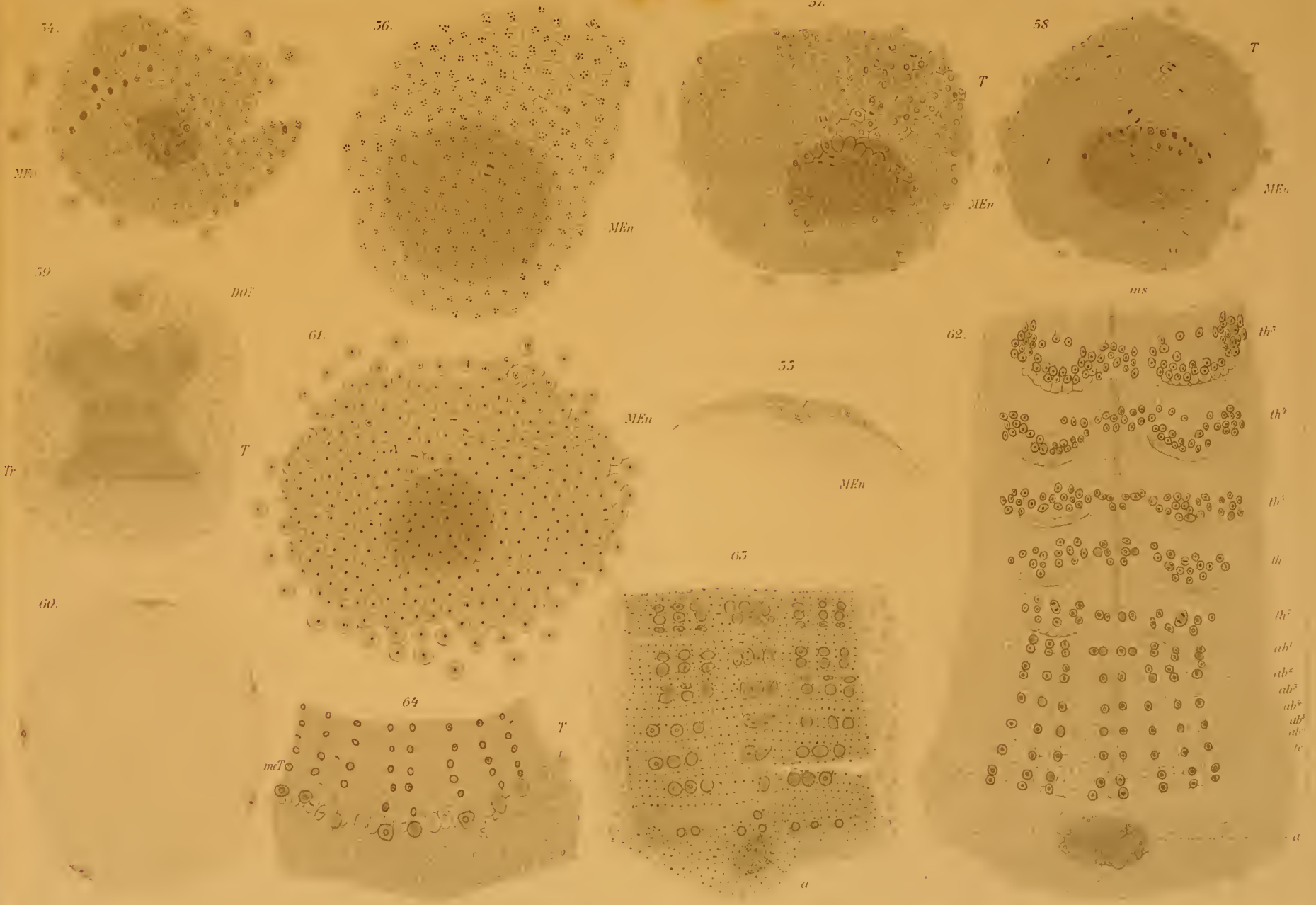
FIG. 60. Section through ovum of *Ligia* at the time when all the cells have reached the surface.

FIG. 61. Blastoderm of *Ligia* at stage when the mes-endoderm cells are sinking beneath the surface.

FIG. 62. Posterior portion of embryo of *Ligia* showing the mesoblasts. *III–VII* = thoracic segments; *1–6* = abdominal segments; *t* = telson.

FIG. 63. Posterior portion of embryo of *Cymothoa* showing the mode of division of the mesoblasts.

FIG. 64. Posterior portion of embryo of *Cymothoa* showing the ectodermal and mesodermal teloblasts.


54.
MEn
56.
MEn
57.
T
MEn
58
T
MEn
ms
59
Tr
60.
61.
T
MEn
62.
th⁵
th⁴
th³
th
th²
ab¹
ab²
ab³
ab⁴
ab⁵
ab⁶
tc
a
63
MEn
a
64
T
mT

EXPLANATION OF PLATE IX.

FIG. 65. Section through the thoracic region of an embryo of *Cymothoa* in a stage somewhat later than that represented in Fig. 62.

FIG. 66. Section through the second thoracic appendage of an embryo of *Cymothoa* older than that from which Fig. 65 was taken.

FIG. 67. Section through the abdominal region of an embryo of *Cymothoa* in which the yolk is beginning to disintegrate. *cn* = chromatin nebula.

FIG. 68. Section through the thoracic region of an embryo of *Cymothoa* in which the disintegration of the yolk is complete.

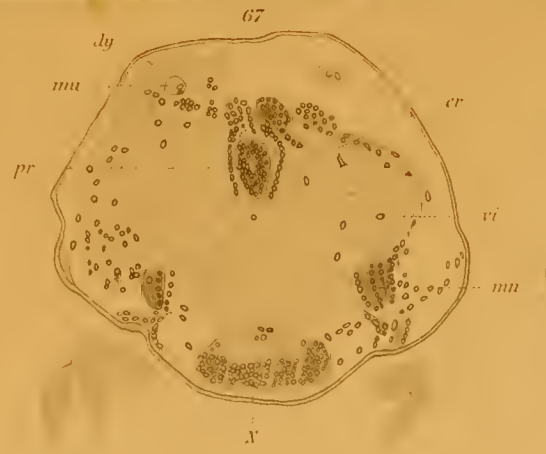
FIG. 69. Anterior portion of an embryo of *Cymothoa.*

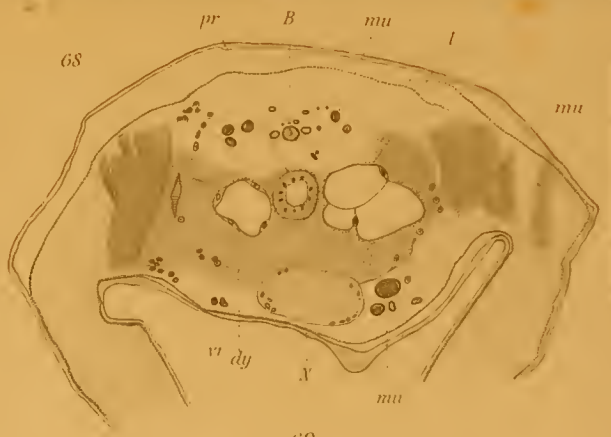
FIG. 70. Anterior portion of an embryo of *Jaera.*

FIG. 71. Lateral view of an advanced embryo of *Jaera.*

FIG. 72. Lateral view of an embryo of *Jaera* almost ready to hatch.


65.
th
cM
X
mM
th
lM


67
dy
mu
cr
pr
vi
mn
X

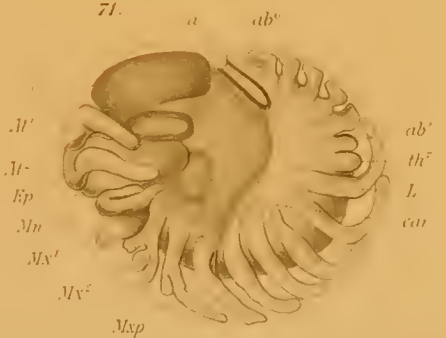

pr
B
mu
l
68
mu
vi
dy
X
mu

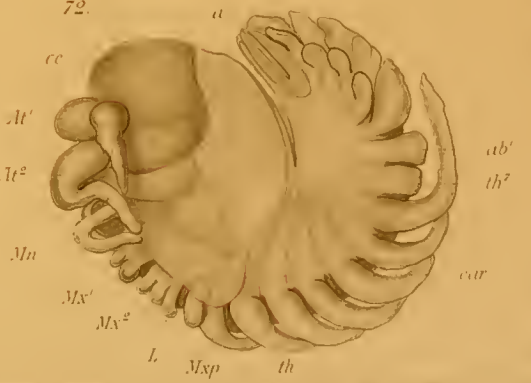
69


66
th'
X
Mxp
mM
th
lM


70
cc
op
G
aG
cp
M'
atG
M²
mG
mt
Mn
L
Mx
Mx²
Mxp
th²
th³


op
G
cc
aG
M
atG
st
mG
M²
L
Mn
Mx'
Mx²
Mxp


71.
a
ab'
M'
ab'
M²
th²
Ep
L
Mn
car
Mx'
Mx²
Mxp


72.
a
cc
M'
ab'
M²
th²
Mn
car
Mx'
Mx²
L
Mxp
th

EARLY DEVELOPMENT OF POLYCHOERUS CAUDATUS, MARK.

EDWARD G. GARDINER, PH.D.

THIS worm, first described by Mark (1), may be found in abundance in the waters of Woods Holl harbor and vicinity. During the summer months it lives on the eel-grass and on dead shells in shallow water. In the northwest gutter of Hadley Harbor, where there are extensive sand and mud flats covered by but a few inches of water at low tide, large numbers of dead shells of clams, pectens, and mussels are scattered over the surface. On the under and thus darkened side, these beautiful little red worms collect in quantity, often as many as a dozen on one shell. That they prefer the dark is evident even when placed in a clear glass dish on a table near the window, as they invariably congregate on the surface away from the light ; or if a small portion of the dish is shaded they seek the darkest corner, crowding close together and often one on top of another to secure a position sheltered from the light. Also if shells are placed in the dish they cluster on the lower and thus shaded side of the shell. They seem to prefer a clean, fresh shell, and are to be found more frequently on the shaded surface of a white clam shell than on that of those darkened by age and marine growths.

It is on these sheltered portions of their favorite abiding places that the eggs are to be sought, for when kept in a glass dish they lay their eggs quite freely. It is noticeable, however, that the eggs are more frequently deposited on the under surface of a shell, if there be one in the dish, than in the light ; also that they lay more frequently in the night than in the day-time if no place sheltered from the light rays is offered to them. That it is darkness rather than the hour of night which induces them to deposit their ova, is shown by the following experiment at the season when they were laying

most abundantly. Two dishes of salt water, both containing these Turbellaria, were placed side by side near the window in a well lighted room, and one of these dishes was covered by a dark felt hat. At the end of the afternoon nearly twice as many clusters of ova were found in the darkened dish as were in the dish exposed to the light.

The process of ovulation has frequently been watched with a lens when the ova were being deposited on the side of a finger-bowl or small dish. When the individual is about to deposit its ova, it draws in all projecting portions of its body, assuming the form of an oval disk so that at the first glance it is difficult to distinguish the anterior from the posterior end. Then the central part of the body is elevated so that a space is left between the body and the glass. As the ova pass out of the female genital pore they appear at first unsurrounded by any capsule, but in the course of a few minutes they appear to be contained in a clear transparent mucus mass. From two to ten ova are thus deposited in each capsule. They are never deposited in rows, but seem scattered irregularly through the central part of the capsule, which is of much firmer consistence on the surface than in the interior.

Not infrequently the whole mass rotates several times before it adheres to the glass. In capsules which are deposited on the surface of disintegrating shells the upper surface is often rendered partly obscured by the presence of fine particles of the shell which adhere to the surface of the capsule during this rotation. On one occasion a capsule was deposited on the side of a glass dish where some finely powdered carmine had been smeared, and the result was that the whole surface of the capsule was covered with the fine colored particles.

During the process of ovulation the animal is reluctant to move, and on being disturbed with a needle or other instrument contracts and shrinks from the contact, but leaves the place it occupies much less willingly than under ordinary circumstances. When the disturbance is persisted in, the animal moves off, trailing after it a line of mucus and eggs. Eggs deposited thus unprotected by the full capsule seldom develop normally.

The capsule is soluble in weak hydrochloric and nitric acids.

Sections through the animal containing mature and nearly mature ova show that a small, inconspicuous polar globule spindle is formed some time before the ovum is laid. In some cases two polar globules may be seen after the ovum has been laid, adhering to its surface on the macromeres *A* or *A'* close to the point where the second cleavage plane intercepts the first. While the ovum is still within the parent, and shortly after the polar globules have been formed, a remarkably large and distinct spindle is formed, which, since it appears directly after the formation of the polar bodies, must be the first segmentation spindle. It may be readily seen by placing the animal under a cover slip and by drawing off the excess of water with filter paper, so that the cover slip exerts sufficient pressure to cause the animal to fully expand, but at the same time does not rupture it. In ova examined in this manner the nucleus may appear as a round almost transparent spot of good size in the center of the ovum ; in other ova it is somewhat elongated, while in others it appears as a large dumb-bell-shaped structure (Fig. 31) which occupies the greater part of the ovum. Indeed, the elongation of the nucleus, the formation of the spindle, and drawing apart of the polar suns, may be observed in one specimen examined at intervals of a few hours.

That spindles presenting this peculiar appearance are common in the ova of many Turbellarians is shown by von Graff (2), who in his monograph figures individuals belonging to three different *genera*, in all of which ova containing such spindles are shown. He does not, however, discuss this matter in the text.

A very remarkable phenomenon occurs in connection with this spindle ; for if the animal be kept too long under the cover slip or placed under certain abnormal conditions, the polar suns grow dimmer and draw closer together and the dumb-bell-shaped structure disappears entirely until the nucleus appears to return completely to its resting stage, and remains unchanged till after the ovum is laid, when again the spindle is formed, heralding in this case the first stage in segmentation. The formation of the polar globules, the fertilization of

the ovum, the formation of the first spindle and its disappearance under certain conditions, present many problems of interest on which I am now at work, and which will form the subject of a future paper.

Selenka (3) first called attention to a spindle in the uterine ova of *Thysanozoön Diesingii*, which from the description given resembles very strongly, in size and general appearance, that just described in the ova of *P. caudatus*. He describes in detail the formation of the spindle seen by him, and states that after the equatorial plate of chromosomes is formed, the polar suns fade in distinctness and draw closer to one another, while the chromatosomes melt together, and the whole nucleus returns to its resting stage. Selenka suggests that this partial karyokinesis occurs in order to effect a rearrangement of the yolk particles. Lang (4) states that among the Polyclads, he has observed even in the uterine eggs a similar phenomenon, but cannot accept Selenka's explanation as to the object for which this spindle is formed, for no such rearrangement of the yolk globules occurs at this stage. Wheeler (5) has also observed a similar spindle to occur in the uterine ova of *Planocera Inquilina*, and also describes it as disappearing before oviposition. All of these authors state that in these Polyclads, the polar bodies are formed and the fertilization of the ovum effected *after oviposition;* therefore the spindle in these forms cannot be the same in origin as that just described in *Polychoerus*, in which form the polar bodies are formed *before oviposition*.

The early stages of segmentation may be studied without removing the ova from the capsule, by placing the whole capsule in a concave slide. This method is, however, unsatisfactory, for it is impossible to rotate the ovum under inspection. When taken from the capsule, which is easily done with needles under a dissecting microscope, the ova generally segment abnormally, and soon die. All of the ordinary killing reagents such as Perenyi, corrosive sublimate, and chromic acid in its various combinations as recommended by different authors, are perfectly useless for these ova, for any of these reagents destroy all trace of segmentation. The most effective

reagent is a mixture of equal parts of absolute alcohol and glacial acetic acid, and even this is very uncertain in its effects, some ova being fixed and losing their color almost immediately, while others are affected very slowly. Examination shows that in these latter the cell structure is much injured, or, more generally, totally destroyed. The reason for this uncertainty is probably due to the difficulty of removing all of the mucus capsule by which they are protected from the salt water. The inner part of this capsule is so transparent that its presence can hardly be detected.

Ova which have been cut out of the parent are of a delicate yellowish white color, and no trace of pigment is to be seen in them. When laid they are about .06 × .04 mm., and a few flecks of a reddish yellow pigment may be seen scattered over the surface. Examination with an oil immersion shows that these pigment granules are between 2 and 3 μ. in size, and are, roughly speaking, double spheres in shape. Fig. 32 represents these granules, *a*, seen from the side, and *b* from the end. The form, as well as the curious movements and change of position which they undergo, suggest that they are some form of alga, but I have not been able to demonstrate that such is the case.

In the newly laid ovum they are few in number, but may be seen, even with a fairly low power, lying on or near the surface of the ovum. After the ovum is laid they begin to multiply and apparently to migrate from within toward the surface of the cell. As will be related in the description of the early segmentation, they form a girdle about the ovum which heralds the first cleavage, and in every successive cleavage up to the ten-cell stage the line through which the cleavage plane will pass is thus marked out by pigment granules before the cell divides. Nusbaum (6) has shown that the pigment granules in the cells in the tail of the tadpole are moved about in a somewhat similar manner, always moving or being moved, within the cell in which a spindle is forming, in such a manner that they form an equatorial plate in the same plane as the chromatosomes. Further, when the cell divides, the surfaces of the two daughter cells lying in contact are covered with

pigment granules while the rest of the cell is free from them. The action of these granules in the ova of *Polychaerus* is apparently very similar, but unfortunately they are entirely dissolved in alcohol, so that the history of their movements within the cell is beyond reach by sections. Not infrequently, while examining the surface of an ovum with an oil immersion, I have seen one of these granules come up from within the ovum to the surface, and move across the field of vision, but I have never yet seen a granule pass from one cell into another; yet they disappear in some cells and later on appear in quantity in others. When the ovum is so viewed it is clearly suggested that there are wonderfully active forces at work within, for the surface fairly scintillates with movements of the protoplasm and these pigment granules. The manner in which the granules are moved about from place to place indicates the powerful nature of the force within. In regard to the disappearance in one part of the ovum and reappearance in another, Figs. 2, 3, 4, and 5 show these granules massed in cells B, B', C and C', while later on these same cells (Figs. 11 and 12) are almost devoid of them, while on the directly opposite pole of the ovum they are so crowded that the cleavage lines are almost obscured. Fig. 13 shows the pole at which the granules were first massed, and Figs. 14, 16, and 18 show the opposite pole, which at an early stage was almost destitute of color. Fig. 9 gives the appearance of this pole when the granules first collect there, and also shows the color-effect produced when they are closely packed together. That the granules actually migrate from one cell to another seems impossible, for, as is shown in Figs. 23, 24, and 25, the cells are bounded by clear, distinct membranes through which the granules would have to pass. It seems much more probable that they fade out and become disintegrated in one part, while others are organized in other parts.

If an ovum is crushed they begin to fade almost immediately and in a few minutes are no longer distinguishable. When the segmenting ovum has reached the stage shown in Fig. 22 the pigmentation is almost confined to the few cells A, A', B_3, B'_3, and E_3, E'_3, and may be said to have reached its maxi-

mum. With a very low power lens this stage is easily distinguishable by its red spot, which becomes later a little more diffused, and then gradually fades out before the embryo is formed.

The pigment granules which characterize the adult, as described by Mark (1), are of a totally different form and color, and bear no resemblance whatever to those which occur in the ovum. I have examined many animals by crushing them under a cover slip, but have found nothing resembling these pigment granules. If they occur at all in the adult, which I doubt, they are very few in number and therefore difficult to find. So, also, in the immature ovum these pigment granules are rare if they occur at all. The young worm just hatched is marked by granules similar to those formed in the adult, while none or almost none of the above described granules are to be found in it.

In the ova of an undescribed species of *Aphanostoma* (?), which is found in this vicinity, and to which my attention was first called by Professor H. C. Bumpus, a precisely similar arrangement and movement of pigment granules, if they be pigment granules, occurs. They are distinctly larger and redder than those found in the ova of *Polychoerus*, and the dividing line between the two halves (Fig. 32, *a*) is more distinctly marked. This *Aphanostoma* (?) is a dark green color, and no red pigment is to be found in it, and the ova when newly laid, have a color very similar to that of the parent. Soon, however, the red pigment appears on the upper pole, and as segmentation progresses is finally collected on the opposite pole, forming the red spot similar to the one just described, but more brilliant in color. Experiments similar to those detailed by Haberland (7) to prove whether these granules might not be independent organisms, were attempted, but the results were perfectly negative. The ova died very soon and the pigment granules disappeared entirely.

A very remarkable phenomenon during the segmentation of the ova of *Polychoerus* is the manner in which the ovum changes its form and becomes distorted, as if some internal forces were pressing in different directions; also the marked difference

in shape at times when the karyokinetic activity is at its highest as compared to that when the ovum is in its resting stage. The ovum shown in Fig. 10 soon rounds up so as to be indistinguishable from that shown in Fig. 12. Not infrequently, however, one part will round up some time before the other, suggesting most strongly the absence of the antero-posterior and bilateral symmetry which characterizes this form of segmentation.

To turn now to the segmentation of the ovum. Shortly after the ova are laid, the pigment granules form a complete girdle round the smaller axis of each ovum (Fig. 1) and mass themselves also in the region where the cells (*C*, *C'*) will be formed, and then the ovum divides into two cells of equal size (*A* and *A'*). The second cleavage plane is in the region of this pigmented area. It is at right angles to the first and divides the cells *A*, *A'*, into *A*, *B*, and *A'*, *B'* (Fig. 2). Immediately after these cells (*B* and *B'*) have been budded off, they begin to rotate from left to right, so that *B*, instead of lying directly above *A*, and *B'* directly above *A'*, both *B* and *B'* cover the line of the first cleavage plane and rest on both *A* and *A'* (Fig. 3). Fig. 4 shows the position attained by *B* as seen from the side, while *B'* passes to the opposite side of the ovum. After this four-celled stage has been reached a period of rest follows, the ovum becomes again oval, and all cell outline disappears. If it were not for the bright girdle of pigment and the massing of the granules in the neighborhood of *B* and *B'*, the ovum might easily be mistaken for one just laid and unsegmented.

The appearance of the third cleavage plane is preceded by the reappearance of the cell outline and also by a massing of the pigment granules on the surface of *A*, *A'* in the neighborhood of *B* and *B'*, which cells move apart to right or left so that the line of the first cleavage plane may be seen between them. At the same time the cells *C* and *C'* are budded off from *A* and *A'* (Fig. 5), exactly as were *B* and *B'*. No rotation, however, takes place, but the cells *C* and *C'* round up and draw closer together in such a manner that *B* and *B'* are forced still farther apart and come finally to lie on opposite sides of the ovum, as is shown in Figs. 6, 7, 8 and 11.

Again follows a period of rest and disappearance of all cell outlines. Renewed activity in cleavage is heralded by changes in the pigmentation, and the line of the next cleavage plane is indicated by a distinct line of red granules dividing off the upper portions of A and A' in the same direction as that of the second and third cleavage planes and presently D and D' are budded off from A and A' respectively (Fig. 7).

After the usual period of rest, the fifth cleavage plane is, as usual, first indicated by the pigment granules, and then the cells E and E' are budded from A and A' respectively (Fig. 8), and in a plane parallel to the first but at right angles to all other cleavage planes which have been described. Hence we have a ten-celled stage, eight of which cells have been budded off from the original macromeres A and A', which exist now as mere remnants. At this stage we have the cells which will form the germinal layers already differentiated, for a little later, by a process which will be described A and A' pass into the centre of the ovum and form the mesendoderm, while the lineal descendants from B, B', C, C', D, D', E and E' form the ectoderm.

Thus far the segmentation has proceeded with great regularity, but from now on the order in which the cells divide varies so much that it is often difficult to determine exactly in which generation certain cells are to be classed. Although the bilateral symmetry is on the whole maintained, it frequently happens that on one end or side, the cells divide more rapidly than elsewhere, so that an ovum presents the appearance of irregularity; but when this occurs, the cells on the corresponding end or side divide in an exactly similar manner shortly afterward, so that the symmetry is soon regained, and the general scheme of segmentation is always the same. In order to make the description of the process more clear a short explanation of the manner in which the cells are lettered may be necessary. The cells A and A' are the result of the first division of the ovum, and from these B, C, D, E and B', C', D', E' are given off respectively. In all cases the sign $'$ indicates that the cell is descended from A' and its absence that it is descended from A. Thus we have $E_1.r_1$ descended from A

through E, and $E'_{1}.r.1$ descended from A' through E'. All cells marked by letters bearing the sign $'$ lie on the right of the shorter axis as viewed in the figures, except B' and its descendants. The cells B and B' originated from A and A' respectively, and when first formed B was to the left and B' to the right (Fig. 2), and though in a later stage they have come to lie directly on the *shorter axis*, the letter B is still applied to the cell which was budded off from A, and B' to the cell budded off from A'.

The cell B divides subsequently into B_1 and B_2. B_1 will divide into $B_{1.1}$ and $B_{1.2}$, and B_2 will divide into $B_{2.1}$ and $B_{2.2}$. The derivatives from B' on the opposite side of the ovum will be numbered similarly, but all marked with the sign $'$. The cells C, D, E, C', D', and E' are, however, so placed that in the subsequent cleavage some cells derived from each of them will lie on the right and some on the left of a line drawn through the *long axis* of the ovum. The cells lying on the right will be distinguished by an r, and those to the left by an l; thus Cr and Cl, Dr and Dl (Fig. 13). As these cells further divide, those derived from them will be indicated by numbers which will show the generation to which they belong; thus, Dr divides into Dr_1 and Dr_2. In the next generation Dr_1 gives rise to $Dr_{1.1}$ and $Dr_{1.2}$; while Dr_2 divides into $Dr_{2.1}$ and $Dr_{2.2}$.

The system by which the cells arising from E and E' will be distinguished, necessarily differs slightly from that applied to B, C, D, and B', C', D', for E and E' do not give rise to cells which lie to the right or left of the long axis till a somewhat later stage. In the first generation these cells give rise to cells E_1, E_2, E_3, E_4, and E'_1, E'_2, E'_3, E'_4, some of which lie directly on the line of the longer axis which will divide the ovum into right and left halves (Figs. 14, 15 and 16). In the next generation, however, when certain of these cells divide into right and left, they will be distinguished by the letters r and l; thus E_2r and E_2l (Fig. 18).

To return now to the process of segmentation when the ten-celled stage has been reached. As a rule the next cells to divide are B and B', although it often happens that their division is delayed till later. In either case, whether they divide now or

after D and D', E and E' have divided, they invariably give rise to B_1, B_2, B'_1 and B'_2 respectively, as is shown in Fig. 10. This figure shows the pigment granules dividing the cell B into approximately equal parts, and the plane of cleavage follows this line. Occasionally the line of granules and cleavage plane is somewhat oblique to the line as represented in this figure, and in such cases a rotation takes place, so that eventually the cells B_1 and B_2 lie as is shown in Figs. 15 and 20. The cells B'_1 and B'_2 are derived from B' in an exactly similar manner, and lie on the side of the ovum opposite to that drawn. At about the same time B_3 and B'_3 are budded off from B_2 and B'_2 respectively.

The cells D and D' are generally the next ones to divide. Figs. 8 and 10 show the cell D from the side, and Fig. 11 shows both D and D' from above. The division takes place, as is shown in Fig. 13, along the long axis of the ovum, thus giving rise to Dr, Dl, $D'r$, $D'l$. Also C and C' divide in a similar manner (Fig. 13), giving rise to Cr, Cl, $C'r$, $C'l$. At about the same time as this takes place, E and E' divide in such a manner that we have E_1, E_2, and E'_1, E'_2, as is shown from the lower pole in Fig. 14 and from the side in Fig. 15. In Fig. 14 it will be seen that the pigment in E_2 and E'_2 is massing close to the cells A and A', and this heralds the formation of E_3 and E'_3 which are shown in Fig. 15 from the side and in Fig. 16 from below. In this latter figure it will be noticed that E_3, E'_3, B_3, and B'_3 form as it were a cross on the lower pole, with the cells A and A' in the center of it. In Fig. 17 (the same stage as shown in Fig. 16) the ovum is viewed from the lower pole and drawn as if transparent. The cells E_3, E'_3, E_2, E'_2, B_3, B'_3, A and A' lie on the surface and partly conceal E_1, E'_1, B_1, B'_1, B_2 and B'_2, which lie below them, while Dr, Dl, $D'r$, $D'l$, Cr, Cl, $C'r$, $C'l$ are completely hidden and indicated by dotted lines.

Presently the cells E_1, E_2, E'_1, E'_2, divide in a plane parallel to the long axis of the ovum so that E_1r, E_1l, E_2r, E_2l, E'_1r, E'_1l, E'_2r, E'_2l are formed as is shown in Fig. 18. The relative position of these cells is best shown in Fig. 19, in which the ovum is again treated as if transparent. Almost

coincident with these changes the cells E_4r, E_4l, E'_4r, E'_4l are formed. Fig. 20, which is a view of the right side of the ovum, shows E_4r and E'_4r, and in Fig. 21, which is a diagram of this stage, and treated as if transparent, E_4l and E'_4l are shown by dotted lines. Apparently these cells arose from a division of E_1r, E_1l, E'_1r, E'_1l respectively.

After this the ovum having attained a thirty-eight-celled stage goes into its characteristic resting stage and the cell outlines become obscure. During the next period of activity the ovum reaches a sixty-six-celled stage, apparently in the following manner. It is, however, almost impossible to identify with absolute certainty the order in which the cells divide. It appears, however, that Dr_1 divides so as to give rise to $Dr_{1.1}$ and $Dr_{1.2}$ (Fig. 22 and 26); Dr_2 into $Dr_{2.1}$ and $Dr_{2.2}$; Dr_3 into $Dr_{3.1}$ and $Dr_{3.2}$; Dl_1 into $Dl_{1.1}$ and $Dl_{1.2}$; Dl_2 into $Dl_{2.1}$ and $Dl_{2.2}$; Dl_3 into $Dl_{3.1}$ and $Dl_{3.2}$; $D'r_1$ into $D'r_{1.1}$ and $D'r_{1.2}$; $D'r_2$ into $D'r_{2.1}$ and $D'r_{2.2}$; $D'r_3$ into $D'r_{3.1}$, $D'r_{3.2}$; $D'l_1$ into $D'l_{1.1}$ and $D'l_{1.2}$; $D'l_2$ into $D'l_{2.1}$ and $D'l_{2.2}$; $D'l_3$ into $D'l_{3.1}$ and $D'l_{3.2}$; making in all twenty-four cells derived from D and D'. The cell E_1r divides into E_1r_1 and E_1r_2; E_2r into E_2r_1 and E_2r_2; E_4r into E_4r_1 and E_4r_2; E_1l into E_1l_1 and E_1l_2; E_2l into E_2l_1 and E_2l_2; E_4l into E_4l_1, E_4l_2; E'_1r into E'_1r_1 and E'_1r_2; E'_2r into E'_2r_1 and E'_2r_2; E'_4r into E'_4r_1 and E'_4r_2; E'_1l into E'_1l_1 and E'_1l_2; E'_2l into E'_2l_1 and E'_2l_2; E'_4l into E'_4l_1 and E'_4l_2. The cells E_3 and E'_3 have not divided, but including them the total of the derivatives from E and E' is twenty-six cells. B_1 also divides into $B_{1.1}$ and $B_{1.2}$; B_2 into $B_{2.1}$ and $B_{2.2}$; B'_1 into $B'_{1.1}$ and $B'_{1.2}$; B'_2 into $B'_{2.1}$ and $B'_{2.2}$, which together with B_3 and B'_3 make ten cells derived from B and B'.

Fig. 26, which is a diagrammatic representation of the sixty-six-celled stage (Fig. 22), shows the manner in which this stage has been derived. The dotted lines represent the cells on the opposite side of the ovum. It will be seen that I represents the cells A and A' from which B, B', C, C', D, D', and E, E' have been budded off successively. The ten derivatives from B and B' are shown in II; in III are the four derivatives from C and C'; in IV the twelve derivatives from D; in V the

thirteen derivatives from *E* ; in *VI* the twelve derivatives from *D'*; in *VII* the thirteen derivatives from *E'*; making in all sixty-six cells, all, except *A*, *A'*, which are much the largest, of about the same size.

Thus we have, as it were, two very different methods of segmentation shown in different periods during the formation of the sixty-six-celled stage. At first four pairs of cells, *B*, *B'*, *C*, *C'*, *D*, *D'*, and *E*, *E'*, were budded off from the two macromeres *A* and *A'* in successive generations. From now on segmentation progresses by the division of these eight cells, while the remnants of the two macromeres undergo no further division till much later. Further, the first four generations follow always in the order in which they are named (*B* and *B'*, *C* and *C'*, *D* and *D'*, *E* and *E'*) but from now on the generations are less easy to distinguish. For instance, in some cases *D* and *D'* give rise to *Dr*, *Dl*, *D'r* and *D'l* before the cells *E* and *E'* divide at all, while in other cases *E* and *E'* give rise respectively to E_1, E_2, E_3, E'_1, E'_2, E'_3, at the same time or even before *D* and *D'* divide. Hence it is impossible to distinguish with *accuracy* the relative ages of the derivatives of the eight cells. They appear, however, to be formed most generally in the order above related, which is also shown in the diagrammatic cell lineage shown on p. 168. From the appearance of the first cleavage plane on, both bilateral and antero-posterior symmetry are maintained. By subjecting the ten-celled stage ova to pressure by crushing them slowly, and also by sections, a better idea of the relative size of the cells can be obtained than by merely surface views, and it is apparent that *C* and *C'* are the smallest of those budded from *A*, *A'* ; *B*, *B'* are next larger in size, *D*, *D'* next, and *E*, *E'* the largest.

To form the sixty-six cells

C, *C'* have given rise to		4 cells	
B, *B'* "	"	"	10 "
D, *D'* "	"	"	24 "
E, *E'* "	"	"	26 "
			64 cells of about the same size (Fig. 22).
The remnants of the two first macromeres	*A*, *A'* "	"	2 cells larger than the above.
			66 cells.

Beyond this stage I have not been able to follow the cell lineage, for surface views show that all visible parts of the cells are so nearly of the same size and form, that it is impossible to distinguish one from another. It appears, however, as if all

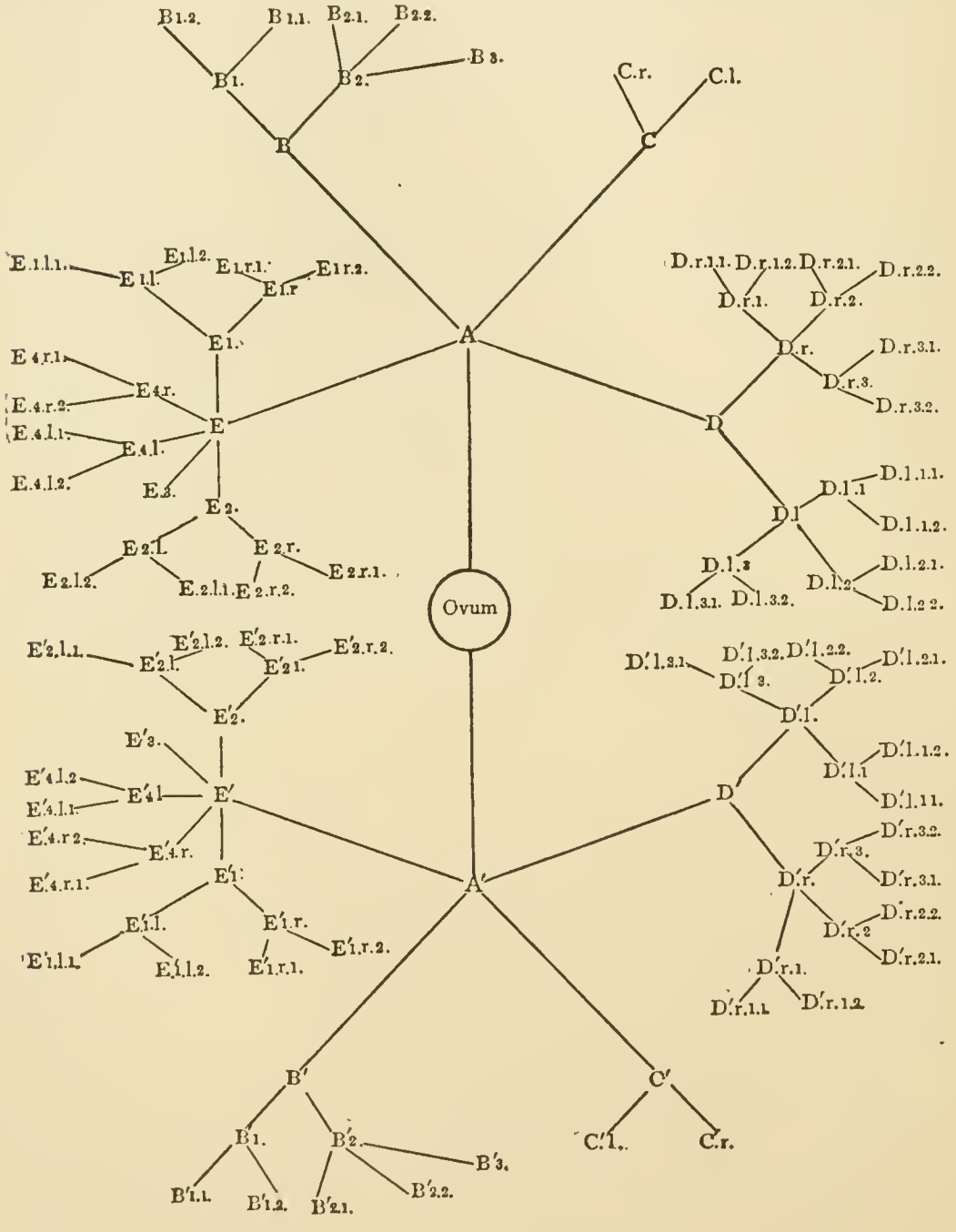


DIAGRAM OF CELL LINEAGE.

cells (except *A* and *A′*) underwent division at about the same time, for soon the whole surface of the ovum is made up of exceedingly small cells.

To understand the fate of A, A' it is necessary to return to the description of the ovum in its earlier stages. When the ovum consists of but four cells a distinct segmentation cavity exists, as is shown in section, Fig. 23. As the ovum returns to its resting stage this cavity becomes obliterated by the rounding of the outer surfaces and consequent sinking in of the cells (Fig. 24). This disappearance of the cavity is, however, merely temporary, for with renewed karyokinetic activity of the cells it is again formed. In the ten-celled stage (Fig. 8) the cavity reappears again in section (Fig. 25), but to be obliterated as before by the sinking in of the cells A, A', and also by the change of form of all the other surrounding cells (Fig. 27), particularly E, E'. At this stage the ovum becomes much elongated and quite deeply arched (Fig. 10), the cells A, A' being always on the concave side.

Fig. 28 shows a horizontal section through the sixteen-celled stage in which no trace of the cavity is to be found, while in the next figure (Fig. 29), a cross section shows a large cavity. This alternate formation and obliteration of the cavity continues until the ovum has obtained the magnitude of upward of sixty-six cells, when the cells A, A' sink into and completely fill the cavity, while the cells B_3, B'_3 and E_3, E'_3 (Figs. 16 and 18) draw together and cover the space in the surface left by A, A'. Sections of later stages show no trace whatever of this segmentation cavity. On the contrary, Fig. 33, a cross section through a distinctly later stage, shows an outer layer of small cells, while within are a mass of much larger cells evidently derived from A, A'. These inner cells form the mesentoderm of the adult, while the outer ones, all descendants of B, C, D, E, and B', C', D', E', form the ectoderm. At a still later stage (Fig. 34) the cells of both mesendoderm and ectoderm are all of about the same size, showing that the division of the cells A, A' and their descendants must have been quite rapid. The ectoderm is already quite differentiated from the central mass. Soon, however, it becomes distinctly differentiated into a two-celled layer, and the central mesentodermic mass seems to undergo a process of degeneration. Open spaces occur, and in the center the cells are less numerous, as is shown in

Fig. 35, a horizontal section through a very advanced embryo. This section suggests in its appearance a larval coelenterate planula of some kind. From the remnants of this mesentodermal mass the parynchym of the body is formed.

No trace of an alimentary tract is to be found at any time during the development, for I have sectioned with great care every stage up to and including the free-swimming young. During the summer of 1894 I obtained the ova of the undescribed species of *Aphanostoma* (?) referred to above and compared its segmentation with that of *Polychoerus*. In every detail the processes are alike in both : the formation of the eight cells by the continuous division of the original macromeres ; the subsequent division of each of these cells so as to form a thirty-eight-celled stage ; the existence of a segmentation cavity and its obliteration by the sinking in of the remnants of the two original macromeres, and throughout all of this the maintenance of the antero-posterior, as well as bilateral symmetry. All of these points have been carefully observed and compared, though no attempt was made to study the later stages of *Aphanostoma*.

The only work which I have seen which treats of the development of any of the Acoela is a paper by Mlle. S. Pereyaslawzew (8) in which she describes the segmentation of *Aphanostoma*, *Nadina*, *Proporus*, *Convoluta*, *Cyromorpha*. She finds that in all of these *genera* the segmentation process is identical. Unfortunately no figures are given in her paper, and it is impossible to follow the process from description alone with absolute certainty. It is apparent, however, that while my observations may differ from hers in a few minor details, yet we agree as to the general plan of segmentation.

W. Repiachoff in a "Nachtrag zu Pereyaslawzew" states that he also has studied the segmentation of the same form as Mlle. Pereyaslawzew described, and finds that his observations with very slight exceptions confirm those made by her. He, however, states that he has seen in the "Archigastrula ähnliches Stadium" "eine deutliche Urdarmhöhle." By "Archigastrula ähnliches Stadium" he probably refers to the stage shown in Figs. 14, 15, 16, and Fig. 27, which latter is a longitu-

dinal section through the ten-celled stage. As will be seen in these figures, the ovum is much arched, and the cells abutting on A, A' appear to be pressing them into the cavity.

"Eine deutliche Urdarmhöhle" doubtless refers to the blastopore-like opening left when A, A' finally pass into the segmentation cavity.

Very shortly after this takes place the cells E_3, B_3, E'_3, and B'_3 close over this depression, and it is completely obliterated except for the pigment which remains for some time. This, however, disappears, and it is impossible to determine whether the mouth of the future embryo is to be formed at this point or elsewhere.

MARINE BIOLOGICAL LABORATORY, WOODS HOLL, MASS.,
September 12, 1894.

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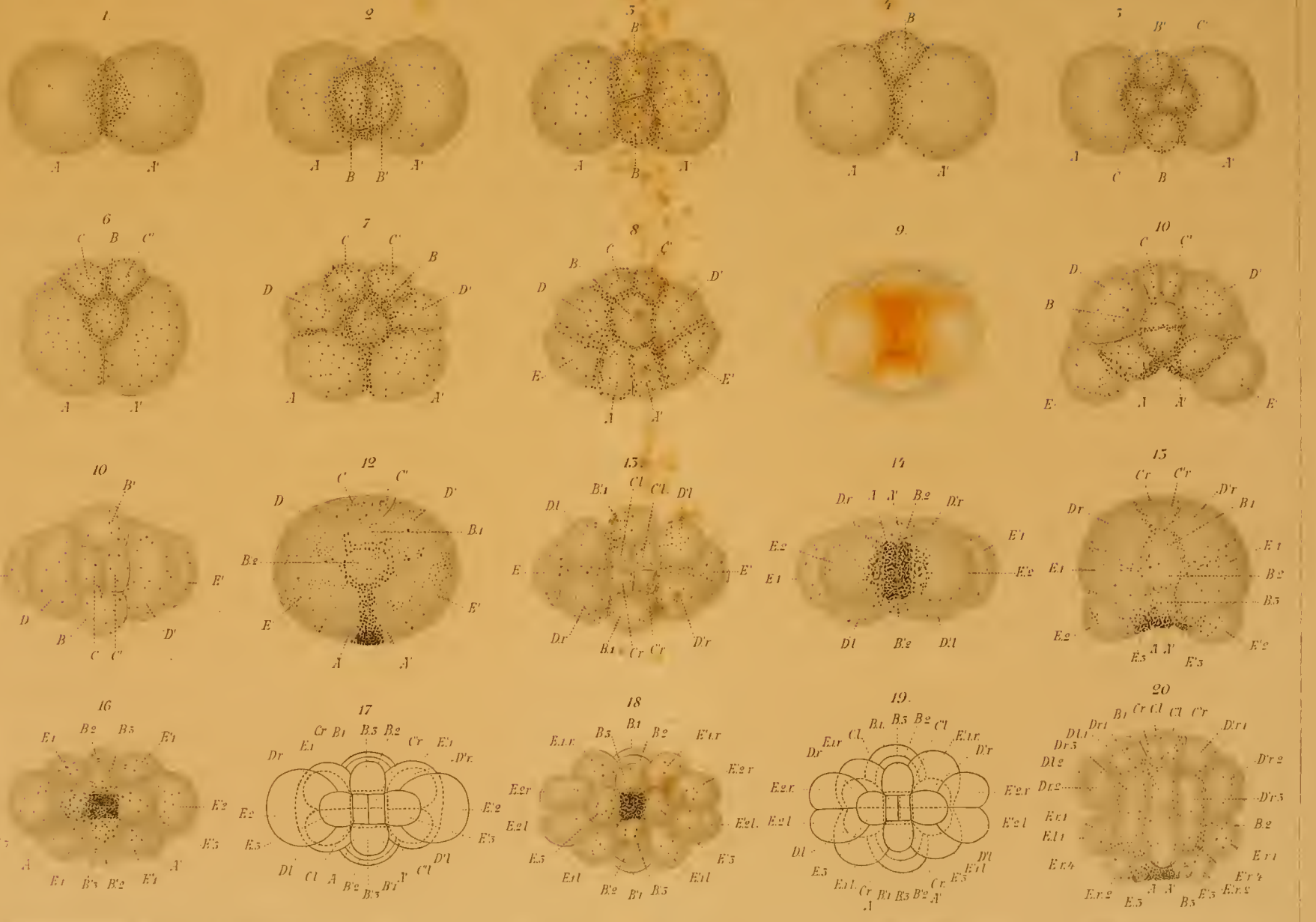
EXPLANATION OF PLATE X.

FIG. 1. Two-celled stage. First cleavage.
FIG. 2. Four-celled stage viewed from above.
FIG. 3. Four-celled stage after the rotation of B and B'.
FIG. 4. Four-celled stage viewed from the side.
FIG. 5. Six-celled stage viewed from above.
FIG. 6. Six-celled stage viewed from the side.
FIG. 7. Eight-celled stage viewed from the side.
FIG. 8. Ten-celled stage viewed from the side.
FIG. 9. Ten-celled stage viewed from below. Natural color. Drawn by R. Tahano.
FIG. 10. Ten-celled stage viewed from the side; the ovum somewhat crescent-shaped.
FIG. 11. Ten-celled stage viewed from above.
FIG. 12. Twelve-celled stage viewed from the side as it goes into its resting stage.
FIG. 13. Sixteen-celled stage viewed from above.
FIG. 14. Sixteen-celled stage viewed from below.
FIG. 15. Sixteen-celled stage viewed from the side; becoming crescent-shaped.
FIG. 16. Eighteen-celled stage viewed from below.
FIG. 17. Eighteen-celled stage viewed from below as if transparent.
FIG. 18. Twenty-six-celled stage viewed from below.
FIG. 19. Twenty-six-celled stage viewed from below as if transparent.
FIG. 20. Thirty-eight-celled stage viewed from the side.


1
A A'
2
A B B' A'
3
B'
A B A'
4
B
A A'
5
B' C'
A C B A'
6
C B C'
A A'
7
C C'
B
D D'
A A'
8
C C'
B
D D'
E E'
A A'
9
10
C C'
D D'
B
E A A' E'
10
B'
E E'
D B D'
C C'
12
C C'
D D'
B 1
B 2
E E'
A A'
13
Cl C'l
B'1 D'l
Dl
E E'
Dr B 1 Cr Cr D'r
14
Dr A A' B 2 Dr
E 2
E 1
E'1
E'2
Dl B'2 D'l
15
C'r C'r
D'r
B 1
Dr
E 1
E 1
B 2
B 3
E 2
E'2
E 3 A A' E'3
16
E 1 B 2 B 3 E'1
E 2
E'2
E 3
E'3
A A'
E 1 B'3 B'2 E'1
17
Cr B 1 B 3 B 2 Cr E'1
D'r
Dr
E 1
E 2
E'2
E 3
E'3
Dl Cl A B'2 B'1 B'3 A' C'l D'l
18
B 3
B 1
B 2
E'1 r
E 1 r
E'2 r
E 2 l
E'2 l
E 3
E'3
E 1 l
E'1 l
B'2 B'1 B'3
19
B 1 B 3 B 2 C'l
E 1 r
E'1 r
Dr
D'r
E 2 r
E'2 r
E 2 l
E'2 l
Dl
D'l
E 3
E'3
E 1 l
E'1 l
A Cr B'1 B'3 B'2 A'
20
B 1 Cr Cl C'l C'r
Dr 1
D'r 1
Dl 1
Dr 3
Dl 2
D'r 2
Dl 3
D'r 3
E r 1
B 2
E l 1
E r 4
E'r 4
E r 2
E 3
B 3
E'r 2
A A'
E'3

EXPLANATION OF PLATE XI.

FIG. 21. Diagram of the thirty-eight-celled stage. The cells on the farther side indicated by dotted lines.

FIG. 22. Sixty-six-celled stage.

FIG. 23. Longitudinal section through the four-celled stage. Showing the segmentation cavity, *S*.

FIG. 24. The same as Fig. 23 during the resting stage. Showing the segmentation cavity obliterated.

FIG. 25. Longitudinal section through the ten-celled stage. Showing the segmentation cavity, *S*.

FIG. 26. Diagram of the sixty-six-celled stage. The cells on the farther side indicated by dotted lines.

I. The remnants of the original blastomeres which form the mesentoderm.
II. The lineal descendants of cells *B* and *B'*.
III. The lineal descendants of cell *C* and *C'*.
IV. The lineal descendants of cell *D*.
V. The lineal descendants of cell *E*.
VI. The lineal descendants of cell *D'*.
VII. The lineal descendants of cell *E'*.

FIG. 27. Longitudinal section through the ten-celled stage. Showing the segmentation cavity filled up by *A* and *A'*.

FIG. 28. Horizontal section through the sixteen-celled stage.

FIG. 29. Cross section through the sixteen-celled stage.

FIG. 30. Horizontal section through the twenty-six-celled stage.

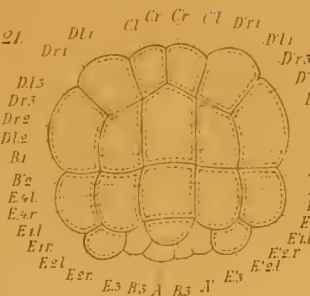
FIG. 31. An ovum cut from the adult. Showing the first cleavage spindle.

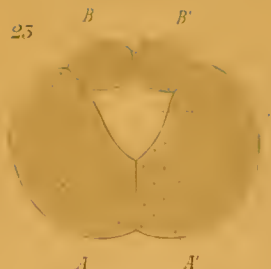
FIG. 32. Pigment granules from an ovum of *Polychaerus caudatus*.

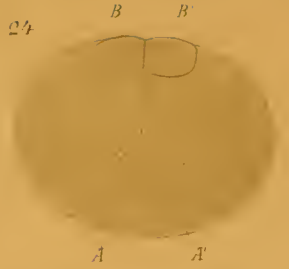
FIG. 33. Section through an ovum much more advanced than the sixty-six-celled stage.

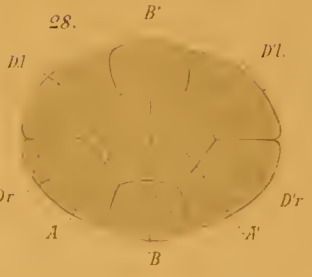
FIG. 34. Section through an ovum still older than that in Fig. 33.

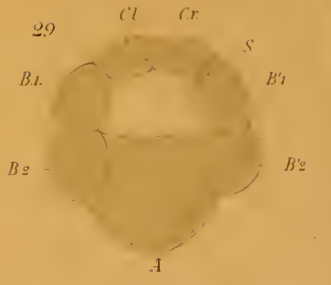
FIG. 35. Horizontal section through a ciliated embryo.

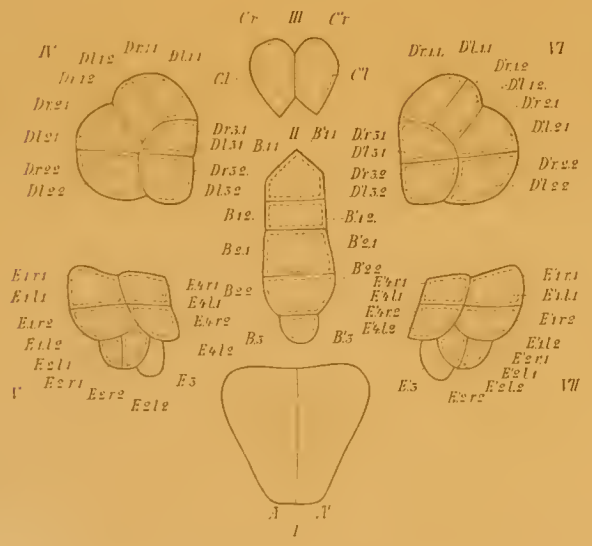


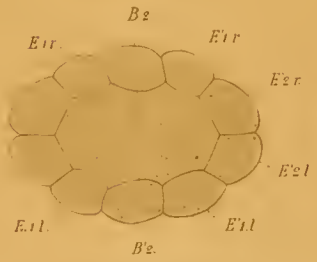


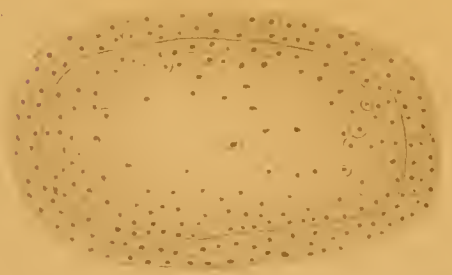


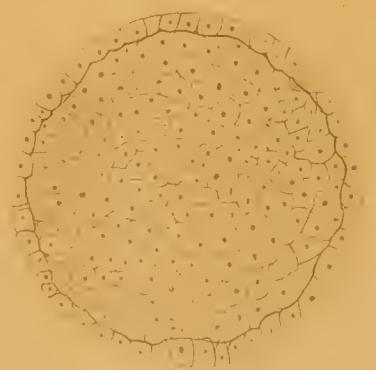












THE PECTORAL APPENDAGES OF PRIONOTUS AND THEIR INNERVATION.

ALBRO D. MORRILL.

THE *Triglidae* have attracted the attention of European anatomists for more than three-quarters of a century on account of the remarkable finger-like processes of the pectoral fins. These processes, which have proved to be free fin rays, were found to be very richly supplied with nerves, and enlargements, or lobes, were found on the dorsal surface of the spinal cord, where these nerves united with it.

Special efforts have been made to discover sense buds or other end organs in the epidermis of these free fin rays.

The strong resemblance to such dermal appendages as barbels, led Merkel (1) to characterize them as wholly analogous in structure and function.

No one has hitherto succeeded in finding sense-organs on these rays similar to those found on barbels, and there are great differences of opinion in regard to the peripheral termination of the nerves in these organs.

In the hope of settling some of these questions, I undertook the study of the Gurnards found along the Atlantic coast.

I wish to acknowledge my great indebtedness to Dr. C. O. Whitman, Director of the Marine Biological Laboratory at Woods Holl, at whose suggestion the work was undertaken, and also to Dr. J. P. McMurrich for many valuable suggestions received during the progress of the work.

The representatives of the Gurnards found along our coast are different from those found in European waters.

Two species which are quite abundant at Woods Holl were studied; namely, *Prionotus palmipes Storer*, and *Prionotus evolans Gill* (14). These fish may attain the length of fifteen to eighteen inches and weigh one and a half to two pounds, but are generally much smaller.

The food of these fish consists of crabs, shrimps, and small fish (14), but they will eat pieces of beef, fish, or shark, and are particularly fond of pieces of clam or snail.

They are very plentiful at Woods Holl during their spawning season, the latter part of May and the first of June. *P. palmipes* occurs in much greater numbers than *P. evolans*.

The pectoral fins, in both species, are very large, being about one-third as long as the body and nearly as broad as long. They extend horizontally from the sides of the body, when expanded, somewhat in the manner of wings, and it is owing to this that they have received the common names of brown-winged and red-winged sea-robins, according to the color of the fins.

They are also known as flying-fish. The term "grunter," which is often applied to them, has reference to the peculiar sounds produced when disturbed.

Nervous System.

The central and peripheral nervous system of both species of *Prionotus* were studied by dissections and macerations.

The differences between the two species are so small as far as the general arrangement is concerned, that a description will only be given of the species figured (*P. palmipes*, Pl. XII, Fig. 3).

The most noticeable feature of the central nervous system and the only part which will be considered here is the series of paired enlargements, six in number, on the dorsal surface of the spinal cord. These enlargements, or "accessory lobes" as designated by Ussow (10), Fig. 3, *ac.l.*, are associated with the origin of the sensory roots of the first three pairs of spinal nerves (I, II, and III). The two anterior pairs of lobes are very indistinct ; in most cases the third pair is well developed, and the fourth and fifth are closely crowded together as if they had at one time been united. The posterior pair is much larger than any of the others. The first spinal nerve (I) arises from the first and second pair of "accessory lobes" ; the second spinal nerve (II) from the third pair, and the extremely large third (III) receives fibers from the last five pairs of lobes.

The sensory root (Fig. 4, *s.r.*) of the third spinal nerve is more than ten times as large as its motor root (Fig. 4, *m.r.*) and extends forward between the motor and sensory roots of the second and first spinal nerves. The motor and sensory roots of the first and second spinal nerves, and the motor root of the third are about equal in size (Fig. 4).

A nerve of the brachial plexus (Fig. 3, *c*) unites the second and third spinal nerves near their origin.

The somewhat flask-shaped enlargements of the first three spinal nerves (g', g'', and g''') just after they arise from the cord, contain the spinal ganglia.

The second spinal nerve (II) passes through a foramen formed by the clavicle and scapula. At the posterior border of the pectoral fin the nerve bends nearly at right angles to penetrate and follow the triangular space between the proximal ends of the paired bones which form the skeleton of each ray and the distal border of the brachial ossicles where the rays articulate with the ossicles. The nerve extends forward and downward nearly to the anterior border of the fin where it unites by cross fibers with the two or three branches from the nerve (Fig. 3, 3) which supplies the posterior free ray. A branch of this nerve (II) is sent to each half of every fin ray (Fig. 3, *n.f.r.* of II) except the two or three anterior ones which are innervated by branches (Fig. 3, *n.f.r.*) from the nerve of the posterior free ray (Fig. 3, 3) as already noted.

The third spinal nerve (Fig. 3, III) passes under the pectoral girdle and divides into three large branches (Fig 3, 1, 2, 3) quite near its origin. Each of these divisions, anterior (Fig. 3, 1), middle (2), and posterior (3), is much larger than the entire trunk of either the first (I) or second (II) spinal nerves. They lie on the inner surface of the muscles of the fin, just beneath the skin.

The middle branch (2) divides into two parts (Fig. 3, A and B); the former innervates the second free ray, counting from the head, while the latter passes to the posterior surface of the third free ray and with the posterior branch (3) of the third spinal nerve (III) supplies that ray. The anterior branch of the third spinal nerve (1) furnishes the nerve supply for the first free ray.

In some cases the middle branch (2) of the third spinal nerve does not divide before reaching the base of the second free ray ; no branch is given in such a case to the third free ray. The nerves supplying the first and second free rays divide in each into two nearly equal rather large nerves and one or two small ones near the base of the ray. The larger nerves lie in the anterior and posterior portions of the rays and break up into smaller branches in their course, which can be traced to the papillated surface of the skin. There is considerable variation in the branching of these nerves.

Morphology of the Free Rays.

The three free rays of the pectoral fins in both species of *Prionotus* have the form of hooked finger-like appendages, the distal third of each being bent almost at right angles to the proximal two-thirds. When the fish is swimming the free rays are held close to the body, and are hidden by the fins viewed from above or at the side. The fish when resting quietly on the bottom of a tank or pool brings these rays to a position parallel to each other, with the somewhat knob-shaped tips near the sides of the head, touching the surface on which the fish is resting and along a line which makes an angle, posteriorly, of about eighty degrees with the long axis of the body.

These rays can be moved through an arc of 180°; a considerably greater freedom of motion than is possessed by the other rays of the pectoral fin. The free rays increase in size from the first or anterior to the posterior, the latter being much longer as well as larger than the first. In *P. evolans*, the free rays resemble normal fin rays to a considerable extent. They have, however, become imperfectly quadrilateral from the angle outward, and are slightly enlarged. In *P. palmipes* there is considerable modification. Distally to the angle of the free ray, it is pentagonal in cross section with a reëntrant angle on the faces which look backward when the ray is in the resting position (Figs. 1 and 2). The anterior face is the narrower and the outer the broader. The breadth of the distal portion of the free ray increases from the angle for about one-third of

its length and then gradually decreases to the end, making this portion of the ray resemble a pair of truncated pyramids placed base to base. Separating the anterior from the outer and inner faces are two narrow ridges (Fig. 1, *r* and *r'*), one on either side, which arise just proximally to the angle of the ray and passing outward gradually increase in height and breadth until they end abruptly in the knob-shaped terminal enlargement (*k*).

The ridges are covered with conical papillae which are closely confined to them until near the tip of the ray, where they spread out over the narrow intervening portion of the anterior face (Fig. 1). Papillae also appear on the outer and inner faces. They gradually increase in number from the posterior edges of these surfaces until they completely cover them a short distance from the tip ; consequently the proximal fourth of the knob-like end of the ray is completely covered with papillae, those nearest the tip being the largest, .15 mm. in diameter. There are no papillae on the faces which form the reëntrant angle (Fig. 2). The size of the papillae varies from .08 mm. to .15 mm. in diameter. The smaller ones are scattered between the larger.

The largest papillae occur, as already stated, at the tips, while others of nearly equal size are found on the ridges separating the anterior from the outer and inner surfaces of the ray. The color of these appendages from the angle to the tip is lemon yellow, but is dotted with many stellate black pigment masses, scattered irregularly over their surfaces.

Skeleton of Free Rays.

The skeleton of each of the free rays consists of two tapering parallel osseous rods, each of which is composed of a great number of semi-transparent, cylindrical bodies joined end to end by thin opaque discs of a cartilaginous substance. Near their proximal ends the skeletal rods become completely ossified, the jointed structure wholly disappearing. The rods, which are arranged dorso-ventrally, diverge near their bases to form a triangular space. They are also slightly enlarged at their points

of articulation with the metapterygial bone. The ventral rod has a flattened conical enlargement on its posterior border for the insertion of the muscles which move the ray. This projection has a height more than twice as great as the diameter of the rod. The halves of the skeleton of the ray approach each other distally. Their inner surfaces are quite firmly bound together by dense connective tissue. Each rod is nearly round in section near its base, but becomes considerably flattened dorso-ventrally in its distal half. The connective tissue in the free rays is very abundant and dense.

Muscles and Blood Supply.

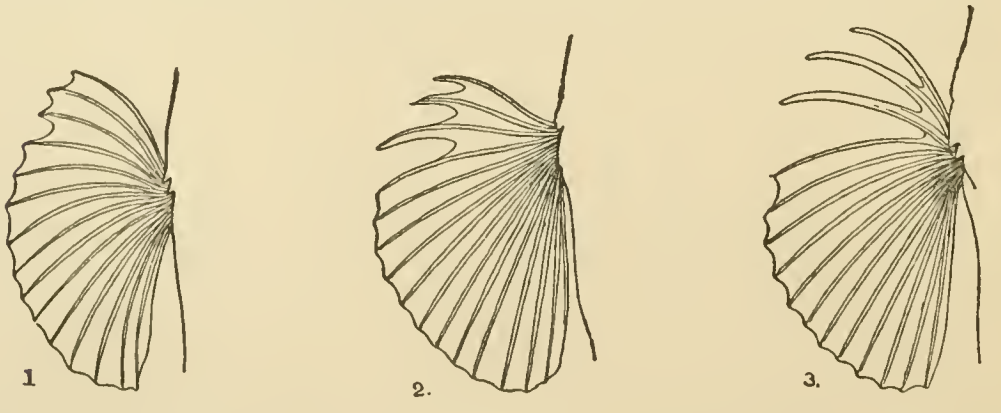
Three pairs of quite large muscles control the movements of each free ray.

The similarity of the musculature to that described in *Trigla* makes it unnecessary to give a detailed description at this time.

The general arrangement of the blood vessels in the free rays will be indicated in describing the cross section.

Development.

In a young sea-robin, probably *P. evolans*, 10 mm. in length, the anterior rays were as yet not in any way distinguished from the others, since their separation from the fin had not begun, being still united to them by the web of the fin. During the



first day after its capture this fish increased 2 mm. in length, and the membrane connecting the three anterior rays had begun to disappear (Fig. 1). The separation began by the

shrinking away of the web between the rays, apparently by absorption. This gradually increased until first the outer parts of each ray were free (Fig. 2). The fish now measured 14 mm. in length. Toward evening of the second day, when the fish was 17 mm. in length, the rays were almost entirely free, only a very small portion of the connecting membrane remained, (Fig. 3) and this eventually disappeared. The process was almost identical in several other specimens which were examined.

Methods.

In studying the anatomy of the nerve-supply of the pectoral appendages, the anterior half of the body was macerated from one to three days, according to the size, in 40% nitric acid. This was rendered necessary by the thick osseous covering of the head of the fish. In the dissection of these acid preparations, porcupine quills were used.

The nerves when freed from the muscles and connective tissue were preserved in 70% alcohol.

For the study of the histology of the free rays, the following reagents gave satisfactory results: Kleinenberg's picro-sulphuric acid, osmic acid, Merkel's fluid, and Müller's fluid. The specimens were washed and preserved in alcohol, passing from 50%–70%, where they remained until used.

The results obtained by the use of Merkel's fluid were in some respects the most satisfactory. The paraffine method was used for obtaining sections which were cut 5 mm. in thickness. The tissues were stained, in mass, with Delafield's haematoxylin or borax carmine. In studying the peripheral terminations of the nerves in these rays, Dogiel's (9) method of using methylen blue gave very suggestive results, but the picrate of ammonia, used to fix the blue, macerated the epidermis so that the ultimate nerve-endings could not be studied as carefully as would have been possible if the epidermis could have been sectioned in place. The best demonstration of the nerve-endings which I obtained, aside from Golgi, was by the use of Mitrophanow's gold chloride method as quoted by Macallum (7). Perfectly fresh tissue was treated with a 1% solu-

tion of gold chloride for one hour, then rinsed in distilled water and placed in a 10% solution of formic acid, and kept in the dark for twenty-four hours. Very little difference was found in the results obtained by treating the tissue first with 10% formic acid for fifteen minutes and then for fifteen minutes with 1% gold chloride. The gold was reduced in this case in a weak solution (2 or 3%) of formic acid in the direct sunlight.

Tissues stained by these methods were imbedded in paraffine by the usual methods, and sections 2 mm. in thickness were cut and mounted in Canada balsam.

The rapid Golgi method gave much more complete demonstrations of the distribution of the nerves in the epidermis than any other method.

Histology.

Cross sections of the free rays of both species of *Prionotus* were made about half an inch from the tip of the ray. In *P. palmipes* the section is somewhat quadrilateral in form, but three of the faces have reëntrant angles, making seven imperfectly marked sides as shown in the outline (Fig. 5).

Covering the epidermis of the free rays is a semi-transparent layer composed of hyaline prisms, each forming a cap over a single cell and probably secreted by it. A similar layer is called the cuticle by Jourdan (4) in his paper on Peristedion. The cuticle is easily separated from the underlying epidermal cells by the action of reagents. It is thinnest over the papillae, and thickest on the surfaces which look backward (Fig. 5 *c*) when the ray is in the resting position. This cuticular layer is very slightly stained by reagents, and shows no nuclei.

The epidermis consists of five or six irregular layers of closely crowded cells. The epidermis will be considered more fully in connection with the nerve endings.

Numerous nerve trunks of various sizes are arranged, as seen in the section (Fig. 5 *n*), nearly parallel to the outer and inner faces of the ray, but lie considerably below the skin. They are closely crowded together around the flattened skeletal bones (Fig. 5, 1 and 2) which occupy the center of the ray. The sections of the parts of the axial skeleton are somewhat crescent-

shaped with their long diameters extending in an antero-posterior direction. The parts of the axial skeleton are separated internally by connective tissue, which holds them firmly in place.

The blood vessels (Fig 5, *b*) are arranged in four groups about the central skeleton, each face having its group.

In *P. evolans* the section is much more nearly quadrilateral in outline (Fig. 6). The same general arrangement of parts exists as in *P. palmipes*, as will be seen from the figures.

Over the papillae (Fig. 7) of the free rays the cells forming the outer layer (Fig. 7, *p*) of the epidermis in *P. palmipes* are somewhat columnar in shape. The outer portion of each cell stains very imperfectly, while the large nucleus which is found near the inner end takes the stain readily.

The inner row of cells (Fig. 7, *b*) has at this portion of the ray been transformed into a layer of cylindrical epithelial cells placed perpendicular to the basement membrane on which it rests. This layer constitutes more than one-third of the entire thickness of the epidermis.

There seems to be considerable uniformity in the position of the nuclei of the outer layer of cells, since all are placed with their long axes transverse to the long axis of their respective cells. In the inner row of cells (Fig. 7, *b*) the long axes of the nuclei and cells coincide. The intermediate rows of epidermal cells have not undergone any modification except that large spindle-shaped, deeply staining cells arranged perpendicularly to the surface of the papillae and broadest on their inner ends are found quite regularly among them, but varying in position.

The epidermis is still more highly modified on the surfaces forming the reëntrant angle. The outer layer has become thickened, and the cells have assumed a spindle form. The inner layer is similar to that found over the papillae, but much thinner, and the intermediate cells have become more than twice as numerous as they are elsewhere on the ray. The spindle-shaped cells are more abundant than over the papillae. There is no sharp line of demarcation between the epidermis on this portion of the ray and that over the papillae, as one passes gradually into the other.

There are no papillae on the surface of the free rays of *P. evolans*. The epidermis closely resembles that already described for the faces of the reëntrant angle in the free rays of *P. palmipes*, except that the spindle-shaped cells are crowded together at certain points (Figs. 8, 9, and 10), and the cuticle is thinner and does not show perpendicular striae.

Nerve Terminations.

The nerves in the free rays form a plexus just beneath the surface of the epidermis (Figs. 7, 9, 10, 13, and 16, *sp.*). The fibers in this plexus are very closely crowded under the papillae in *P. palmipes*, and less so beneath the longitudinal ridges in *P. evolans*. Nerve fibers (Figs. 7, 8, 9, and 10, *nf.*) from the plexus, in both species of *Prionotus*, penetrate the basement membrane and pass out between the cells of the inner layer of the epidermal cells, where they divide, sending numerous branches in all directions along the distal ends of the inner layer of epidermal cells. These fibers soon curve outward, a large proportion of them ending free just below the cuticle, while a smaller number are directly connected with spindle-shaped cells (Figs. 10, 11, 13, 15, 16, and 17) the outer ends of which extend to the cuticle. A single detached cell and nerve fiber is seen in Fig. 17 from a methylen blue preparation.

The gold chloride preparations agreed very closely with those obtained by Golgi's rapid method. The latter (Figs. 12–16), however, showed much greater detail, and brought out the epidermal plexus with great distinctness, a point very imperfectly shown by the use of gold chloride.

The manner of the nerve ending in the epidermis, between the papillae (Fig. 12) in *P. palmipes*, was similar to that over the papillae, except that the nerve fibers were widely scattered and nerve cells rarely found. The peripheral nerve fibers in *P. evolans* were similar to those in the general epidermis of *P. palmipes*, but are fewer in number and more easily studied by the gold chloride method.

Historical.

Dr. Harrison Allen (11) found four layers of epidermal cells in *P. palmipes*, but does not describe the modifications which arise in the epidermis over the surface of the papillae, and does not describe the manner of the nerve endings.

As already noted, the *Triglidae* have received considerable attention from European anatomists. In 1808 Samuel Collins discovered the enlargements on the spinal cord, and in 1811 Tiedeman (12) observed that the free rays were in some way related to the spinal enlargements. The latter also described and figured the musculature of the free rays.

Deslongchamp, who was the first to observe these fish in captivity, claimed that the free rays were organs of locomotion. Tiedeman (12) records having seen a *Trigla* move on the deck of a ship by means of the free rays.

The muscles of the free rays were carefully studied by Deslongchamp, and later by Jobert (2). The latter states that there are many anomalies.

Jobert (2) concludes that the free rays are modified fin rays.

The cross sections of the free ray of *Trigla*, as shown by Jobert (2) and Zincone (3), are elliptical, with the antero-posterior diameter nearly twice as great as the one at right angles to it. The general arrangement of the axial skeleton nerves and blood vessels is essentially the same as in *Prionotus*. No detailed drawings of the epidermis are given.

From the descriptions given by Jourdan (4), Jobert (2), and Zincone (3), the epidermis of the free rays of *Trigla* resembles that in *Peristedion* more than that in *Prionotus*.

There is a sub-epidermal nerve plexus as in *Prionotus*. Merkel (1) was able to trace the nerve fibers into the epidermis, but could not follow them. Jobert (2) by the use of gold chloride, found each fiber connected with a small terminal body (corps épidermique) in the epidermis which measured .004 to .005 mm. in diameter. Zincone (3) claims to have found nerve fibers continuous with spindle-shaped cells.

Jourdan (4) found the non-medullated nerve fibers of the free rays of *Peristedion* terminating in small papillae on the

surface of the dermis insinuated between the cells of the basal layer of the epidermis. In some cases this investigator observed a small terminal enlargement in the papillae, the exact location of which was not indicated.

The drawings of the dorsal surface of the brain and spinal cord of *Trigla adriatica* given by Tiedeman (12), together with his descriptions, show considerable difference between the first three spinal nerves in *Trigla* and *Prionotus*.

The first spinal nerve in *Trigla* is the largest, and arises by three roots, each from the side of an enlargement. The second spinal nerve arises from the fourth enlargement only, while the third spinal nerve arises by two roots, one from the fifth and the other from the sixth enlargement.

The six pairs of spinal enlargements, or accessory lobes (10), are small and appear to be equal in size and distinctness, which is far from being true for *Prionotus*.

Tiedeman (12) found that the fibers of the posterior root of the third spinal nerve were generally very fine, while those of the anterior root were quite coarse. Most of the nerve trunks contained both kinds of fibers, or, as in the case of the branch to the swimming bladder, the fibers were all of the coarser variety. A similar difference in the size of the nerve fibers was observed in *Prionotus*.

Physiology.

When specimens of *Prionotus* were placed in a tank of water in the bottom of which there were several inches of sand, the fish buried themselves in the sand very quickly by a rapid rolling movement as they rested upon its surface. The sand was thrown out on either side and settled over the surface of the body so that in most cases only the eyes, top of the head, and the tip of the nose were visible. Two openings, one on either side of the posterior dorsal portion of the gill-covers for the escape of the water which was taken into the mouth, became visible as the water was forced out.

Little or no attention was generally paid to food for a few moments after it was placed in the water, but as the fish swam over it, as it lay upon the sand or bottom of the tank, the free

rays came in contact with it. The fish immediately began to move the free rays much more rapidly than usual, passing them over the piece of meat or fish several times, and then by a rapid lateral movement snapped it up.

After finding two or three pieces of food the fish were guided by sight, apparently, as they swam across the tank to catch the food before it reached the bottom when fresh pieces were thrown to them. In the large fish ponds of the United States Fish Commission the fish often swam ten feet and were able to secure the food before it reached the bottom, the water being from four to five feet deep. The fish frequently became so excited that any light-colored object was taken into the mouth, such as bones, small pebbles, and pieces of shell. In one case two large specimens of *P. evolans* rushed at the same piece of meat and missing it caught each other's jaws instead.

Inedible objects were quickly dropped. Tainted meat was quickly rejected from the mouth although it might be taken in again almost immediately, only to be thrown out. The same piece of tainted meat was sometimes taken by several fish in succession and then lay unnoticed. Pieces of meat dripping with turpentine were swallowed as readily as the pieces which had not been treated with it.

In order to test the use of the free rays independently of sight the crystalline lenses and cornea were removed from some fish and in other cases the cornea was covered with varnish, balsam, or tar. The repeated experiments were negative in their results, as the fish paid no attention to the food, even when it was placed in contact with the free rays.

Bateson (13) claims that the *Triglidae* do not take food at night. I have not been able to prove that *Prionotus* takes food in darkness.

To test the effect of removing the free rays, fish were selected which took food readily and the free rays were all amputated close to the body in some cases, and in others they were left of different lengths.

When the free rays were all removed the fish occasionally detected food by sight. In one case I saw normal movements of the stumps of the free rays when food fell on the bottom of

the tank near the mutilated fish. The ends of the stumps of the free rays were more than an inch from the food. No effort was made to take the food.

The movements of the free rays of *Prionotus* resemble walking so closely that it is natural that the free rays should be looked upon as locomotor organs. The early observers of the European *Triglidae* held this view. The movements of the free rays are exactly the same when the fish swim upward in contact with the glass sides of the tank as when swimming along the bottom.

These fish have been observed several times to turn over small stones and shells, as they swam along the bottom, by means of the free rays.

The place covered by the stone or shell was subsequently thoroughly examined by means of the free rays, apparently in the search for food.

The method of nerve termination described by Ranvier (8) and other authors (5 and 7), as characteristic of organs of touch is very much like that found in *Prionotus*. This together with the physiological evidence, so far obtained, from the study of the Gurnards, strengthens the opinion that the free rays have been modified for tactile purposes, and that they are mainly if not altogether used in searching for food.

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EXPLANATION OF PLATE XII.

FIG. 1. Anterior surface of free ray of *P. palmipes Storer*, when in the resting position, showing ridges *r* and *r'* covered with papillae and terminal knob *k*. Drawn by R. Takano. × 6.

FIG. 2. Posterior surface of same showing reëntrant angle *a*, marginal papillae *p*, and terminal knob *k*. × 6. Drawn by R. Takano.

FIG. 3. Maceration showing dorsal view of anterior portion of central nervous system of *P. palmipes Storer*, with the branches of the three first spinal nerves, I, II, and III ; *Ol.n.* and *Ol.b.* nerve and bulb ; *Op.n.* and *Op.l.* optic nerve and lobe ; *Cb.* cerebrum ; *cbl.* cerebellum ; *ac.l.* six pairs of accessory lobes on spinal cord ; 1, 2, 3, main branches of third spinal nerve (III) : *A* and *B*, divisions of 2 ; *a* and *b*, principal secondary divisions of 1 ; *c* and *d*, divisions of *A* ; *e*, anterior branch of 3; *fa.* continuation of *B* ; *k*, *l*, and *m*, portions of dermal covering at tips of rays to show distribution of the nerves distally ; *n.f.r.* nerve fibres of rays, one branch being distributed to each half of the ray ; *g*, *h*, *i*, and *j*, branches of 3 ; *c*, a nerve of the branchial plexus connecting second, II, and third, III, spinal nerves ; *g'''*, ganglionic enlargement of the third, III, spinal nerve. × 2.

FIG. 4. Ventral surface of same preparation ; *m.r.* motor root ; *s.r.* extremely large sensory root ; *In.* infundibulum ; *l.i.* lobes inferiores ; IV and V, fourth and fifth spinal nerves. × 2.

FIG. 5. Cross section of free ray of *P. palmipes Storer*, made ½ inch from distal end ; *a*, *p*, *o*, and *i*, anterior, posterior, outer, and inner surfaces ; *c*, thickened cuticle shown on surfaces forming reëntrant angle *r* ; 1 and 2, bones of ray ; *b*, blood vessels ; *n*, nerves. × 30.

FIG. 6. Cross section of free ray of *P. evolans Gill*, ½ inch from distal end. The letters are the same as in Fig. 5. × 30.

FIG. 7. Vertical section of a papilla of free ray of *P. Palmipes Storer ; c*, cuticle ; *b*, proximal layer of epidermal cells ; *p*, peripheral layer ; *sp*, subepidermal plexus ; *nf*, nerve fibers. Obj. 6 Vérick ; cam. luc. Abbe. Gold chloride preparation.

FIG. 8. Cross section of epidermis of *P. evolans Gill.* The parts are indicated as in Fig. 7, *b.m.* basement membrane ; *s.c.* sensory cell. Obj. 6 Vérick ; cam. luc. Abbe ; gold preparation.

FIGS. 9 and 10. Similar to Fig. 8.

FIGS. 11. Sensory cells from *P. evolans Gill*, showing nerve fibers, *nf*, cuticle, *c* ; nucleus of cell *c* and terminal enlargement of cell *t.e.* Gold chloride preparation. Zeiss, oc. 4, $\frac{1}{12}$ oil im.; cam. luc. Abbe.

FIG. 12. Golgi preparation of epidermis of *P. palmipes Storer*, showing distribution of nerve fibers. Zeiss, oc. 4, obj. D.

FIG. 13. Similar to Fig. 12, showing portion of subepidermal and epidermal plexii with sensory cell. Zeiss, oc. 4, $\frac{1}{12}$ oil im., cam. luc. Abbe.

FIG. 14. Similar to Figs. 12 and 13. Oc. 4, obj. D.; cam. luc. Abbe.

FIG. 15. Golgi preparation similar to 12, 13, and 14. *sp*, nerve fibers as they leave the subepidermal plexus. Zeiss, oc. 4, $\frac{1}{12}$ oil im.; cam. luc. Abbe.

FIG. 16. Similar to preceding. Zeiss, oc. 4, obj. D.; cam. luc. Abbe.

FIG. 17. An isolated sensory-cell and nerve fiber from methylen blue preparation.


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THE SENSE-ORGANS OF LUMBRICUS AGRICOLA, HOFFM.[1]

FANNY E. LANGDON.

In December of 1891, sections of Lumbricus agricola, Hoffm.[2] were prepared in the Laboratory of Animal Morphology of the University of Michigan, in which the epidermal cells presented in many places an arrangement similar to that which is found in the vertebrate "taste-buds." At the suggestion of Prof. J. E. Reighard, the writer and Miss M. F. Randolph undertook an examination of the literature and a study of the structure, nerve-supply, and distribution of these apparent sense-organs. Soon after this, there appeared a paper by Lenhossék ('92), in which it is stated that the sensitiveness of Lumbricus is due to *isolated nerve-cells* scattered through the epidermis, and that these nerve-cells are never grouped into sense-organs. An examination of the literature brought out the fact, not referred to in Lenhossék's paper, that epidermal sense-organs had already been seen and described by Leydig ('65), Mojsisovics ('77), Vejdovský ('84), Ude ('86), and Cerfontaine ('90), in Lumbricus agricola, and in other species of Lumbricidae. Our work then resolved itself into an attempt to determine accurately the facts at first hand in order to understand the conflict between the account of Lenhossék and the accounts of earlier writers. As Miss Randolph did not return to the University the next year, I have since carried on the work alone.

For the sake of clearness, I shall give first a continuous account of my own observations, and shall reserve until the end of the paper all discussion of the work of others.

[1] Work from the Laboratory of Animal Morphology of the University of Michigan, under the direction of Prof. J. E. Reighard.

[2] This species, upon which all my work has been done, has the characteristics of L. herculeus, Sav. as given by Ude ('86).

Methods.

Sections stained with Kleinenberg's haematoxylin have been used for a study of the finer structure of the sense-organs and the course of the nerve-bundles passing to the epidermis. The nerve-supply of the sense-organs and the course of the nerve-fibres in the epidermis and in the central nervous system have been studied by means of Golgi's shorter silver nitrate method. The distribution of the sense-organs has been determined from surface views of the removed cuticula.

The fact that these sense-organs of the epidermis have been so often overlooked by competent observers seems to warrant an account of the methods employed by me, although this account contains little that is new.

It is not difficult to obtain well stained sense-organs in a good state of preservation, if sufficient care is taken in all stages of the preparation. To insure successful cutting of sections, it has been found best to feed the worm on wood-pulp in the preparation of which no chemicals have been used. In killing, great care must be taken to avoid contortion or an excessive discharge of mucus from the gland-cells of the epidermis. The method found most successful is the alcohol method given by Cerfontaine ('90), p. 337. But it has been found best to have the alcohol act more slowly; 70% alcohol was used in place of the 96% recommended by Cerfontaine, and it was made to drop on the filter paper at the rate of sixty drops a minute. In about an hour the worms are so stupefied that all the paper may be removed except the small piece on which the alcohol drops, and the alcohol may be made to drop more rapidly. At the end of two hours the worms are placed in 50% alcohol for an hour, in 70% for twenty-four hours — during which this alcohol should be several times renewed — in 96% for twenty-four hours, and then preserved in fresh 96%. It is better to use for sections worms that have been recently prepared. When these preserved worms are cut into pieces, it is best to remove the wood-pulp from the alimentary canal with small forceps in order to facilitate sectioning. Parts of the worm chosen for study are run through absolute alco-

hol, cedar oil, soft paraffin, one-half hard and one-half soft paraffin, and finally embedded in the latter. Each change from one reagent to another must be made *gradually*, and the temperature of the paraffin bath must not rise above 54° C. The minute structure of the sense-organ does not show well in sections cut over 10μ thick.

The serial sections are straightened out and fixed to a slide by the alcohol method. The paraffin is then dissolved in turpentine, and the sections run back into 70% alcohol. They are then stained for about twenty-four hours in a weak solution of Kleinenberg's haematoxylin, prepared according to the formula given in Lee's Vade Mecum. It has been found best to mix the stain about twenty-four hours before using, to use a weak solution, and stain on the slide. When the sections are stained. they are washed in 70% alcohol and then with a saturated solution of sodium bicarbonate in 70% alcohol. If overstained, they may be treated with weak acid alcohol before using the bicarbonate. The sections are then run into absolute alcohol, cleared in clove oil, and mounted in xylol-balsam.

The silver nitrate method used is, in general, that given by Dr. C. J. Huber in *Anat. Anzeiger*, Jahrg. 7 (1892), p. 587, Dr. Huber kindly suggested two changes which add to the permanence of the preparations: first, to leave the turpentine on fifteen minutes instead of five; second, to use pure balsam instead of turpentine-balsam. At the suggestion of Professor Reighard the creosote was replaced by cressylic acid, which is less disagreeable to handle. Care must be taken to remove all traces of the creosote or cressylic acid, otherwise the sections are sure to spoil. For sectioning, pieces of the worm are fastened to a wooden block which can be clamped into the object-holder of a sliding microtome. The end of the block is covered with thin collodion; the object is taken directly from the silver nitrate, in which it can be left until needed, pressed into the collodion, and then covered with a few drops of the latter. If exposed to the air, the collodion will soon harden so as to fix the object firmly in place. It is well to moisten the collodion over the tissue with a little 96% alcohol to keep it from contracting the tissues while it is hardening. When the sections

are cut, the surrounding collodion is easily removed. When it is desirable to use the oil-immersion on these sections, they should be cut about 10μ thick and the cover-glass pressed down *firmly*.

The following method was employed in the preparation of the cuticula for a study of the distribution of the sense-organs. A large worm which has been previously hardened in alcohol is placed in 70%. Beginning at one end, the worm is cut into pieces of 20 metameres each. As each piece is cut off it is placed in 50% alcohol, a longitudinal cut is made with a sharp razor through the body wall in the mid-ventral line, and the edges of the cuticula along this cut are loosened with fine forceps. Then by holding the cuticula firmly at one edge and gently rolling the piece of the worm out from under it, the cuticula is peeled off. All this is done under the 50%. The cuticula usually is ready to peel off about as soon as the piece of the earthworm is placed in the 50%. A glass slide is then held in the alcohol, the cuticula floated onto it, and pressed down with a camel's-hair brush; the slide is removed from the alcohol, and allowed to dry. No cover-glass or further preparation is needed. With a little practice perfect preparations of the entire cuticula may be thus made. Cerfontaine ('90) places the worm in 30% alcohol for three or four days and removes the cuticula entire, afterward cutting out pieces of it for study. This procedure is apt to macerate the cuticula, and the removal of the latter entire renders more difficult the cutting of it into sections for mounting. Cerfontaine says: "La structure de la cuticule ne peut s'observer que sur des préparations fraîches, parce que l'examen doit se faire dans l'eau ou, mieux encore, dans l'alcool." Cuticula dried on the slide as described above has been found to be in perfect condition for study over a year after preparation, and seems to show its structure better than when examined in water or alcohol.

A Study of Haematoxylin Preparations of the Epidermis.

General structure of the epidermis. — The epidermis of Lumbricus agricola is covered exteriorly by a thin cuticula composed of at least two layers of fibres; the fibres of one layer

are at right angles to those of the other layer, and the fibres of both layers are at an angle of 45° to the long axis of the body. The inner surface of the epidermis rests on a thin basement-membrane, which is apparently composed of connective tissue and separates the epidermis from the circular muscle-layer beneath. The epidermis itself is composed of two layers of cells, a superficial and a basal layer, each one cell deep. The cells of the superficial layer are of two kinds, supporting cells and gland-cells. A supporting cell is almost columnar in form; it has a square-cut top, a base prolonged into several processes, and an oval nucleus at about the middle of its height. A gland-cell is "goblet-shaped." It has an external opening through a pore in the cuticula above it for the discharge of its secretion, and the nucleus is usually forced into the base of the cell by the accumulation of the secretion. The base is sometimes broad and entire, and sometimes divided into basal processes.

The cells of the basal layer are imbedded between the basal processes of the cells of the superficial layer. A basal cell is usually rounded and contains a rounded nucleus; sometimes it extends toward the cuticula for a varying distance between the cells of the superficial layer. Most writers state that the supporting cells are probably changed into gland-cells, but Cerfontaine ('90) believes that both supporting and gland-cells are formed from these basal cells. Intermediate stages between the basal cells and both kinds of superficial cells are so easily found as to leave in my mind no doubt as to the correctness of his statement. All the cells of the basal layer rest directly on the basement-membrane. All the cells of the superficial layer which do not rest directly on this membrane reach it by means of their basal processes. (See Cerfontaine ('90) for figures of these various cells.) Imbedded between these various kinds of epidermal cells are groups of sensory cells *collected into definite sense-organs.*

The sense-organs of the epidermis. — The sense-organs in the anterior metameres have been studied as a type (Pl. XIII, Fig. 1). Each organ has in general the form of an ovoid, the smaller end of which projects into a raised spot in the cuticula above it; the broader end is flattened, and rests on the basal mem-

brane of the epidermis. This ovoid may be broad or narrow, and is usually more or less irregular; the base is sometimes distorted by the presence under it of a bundle of nerve fibres. The greatest width is usually just above the base, occasionally it is at the base. In a cross section of a sense-organ its outline rarely appears as a circle, but it is usually a little flattened or otherwise distorted.

The sense-organs in the middle zone of an anterior metamere vary in height from 80 to 100μ. At its summit such an organ may be from 18 to 28μ wide; at the widest part, from 40 to 60μ wide.

The lateral limit of each organ is clearly defined by the layer of supporting cells around it. This is shown in longitudinal sections, in which the sense-organ is clearly outlined on each side by one or two supporting cells, which follow the outline of the organ and which are much flattened, evidently by pressure from within the sense-organ. In a cross section of a sense-organ, the inner (in relation to the organ) walls of these adjacent supporting cells are found to form a continuous membrane around the organ. It is thus seen that the cells of the sense-organs are enclosed in a cavity whose walls are formed by one or two layers of supporting cells which differ from the other supporting cells only in their flattened form. These may be called the *covering cells* of the sense-organ.

Within the receptacle thus formed by the covering cells, lie the two kinds of cells belonging to the sense-organ, small basal cells and long slender cells which extend from the basal membrane of the epidermis through the cuticula. The small cells are sometimes evenly distributed along the base of the sense-organ, and sometimes collected into a little group in the center of the base or toward one side. Each cell is irregularly rounded and almost filled by a rounded nucleus — in fact, it does not differ from the small cells of the basal layer of the epidermis. I have not been able to determine the function of these basal cells of the sense-organ; it seems to me possible that they are the cells which are to produce new sense-cells, but I have found no intermediate forms. The long cells, which are the true sense-cells, occupy the main part of a sense-organ; there

are from 35 to 45 in one of the larger sense-organs. These sense-cells stain about the same color as the supporting cells, but appear more finely granular and differ from them greatly in form. I have found no intermediate forms between the sense-cells and the supporting cells. The supporting cells are either of the same width throughout their height, or slightly wider at base and apex; they have clearly defined cell-walls, and the walls of one cell are closely applied to those of surrounding cells; on account of mutual pressure the outline of a cross section of a cell is nearly hexagonal. The sense-cells, on the other hand, are much narrower at base and apex; in the upper part of each cell it is impossible to distinguish any cell-wall, and it is difficult to see one in the lower part; each cell stands alone, clearly separated from its fellows by a space which appears as if filled by a fluid, and a cross section of a cell is circular or elliptical instead of hexagonal. In a longitudinal section through the center of a sense-organ one might at first suppose these cells had been torn apart in the preparation.

The greater part of the sense-cells have their nucleus at or below the middle of their height; a few cells, usually situated between the center and the lateral surface of the sense-organ, have their nucleus near the cuticula. The part of the sense-cell in which the nucleus lies is always the widest part, and is almost completely filled by the nucleus. If the nucleus occurs near the cuticula, the cell tapers into a very slender, fibre-like base. If the nucleus is found at the middle height of the cell or near the base, the base of the cell does not become so slender, and often sends off several basal processes which pass to the basement-membrane between the small basal cells. Above its nucleus, each sense-cell tapers into a slender part which looks like a delicate strand of protoplasm and reaches to the cuticula. Each cell terminates in a still more slender, hair-like process which passes through a canal in the cuticula and projects stiffly for about 2μ above the surface. The varying position of the nuclei in the sense-cells seems unimportant, and is probably brought about by the cells accommodating themselves to the space in which they lie. The nuclei

generally appear elliptical in longitudinal section; some have a truncated top, and a few have one side concave. In a cross section of a nucleus it appears elliptical, circular, or almost triangular. In each nucleus several large nucleoli and usually a few chromatin threads can be made out. The nuclei of the sense-cells cannot be distinguished from those of the supporting cells.

Over each sense-organ the cuticula is elevated and much thinner than elsewhere. These two features render it concave on the side next to the sense-organ, and convex on the outer surface. In longitudinal sections through a sense-organ, the fine canals through which the sense-hairs pass may be easily seen piercing this place in the cuticula. When the sense-cells are straight and their upper parts quite slender, the hairs show plainly above the cuticula ; but when the upper parts of the sense-cells are thickened or thrown into sinuous curves, the hairs are found to be partly or wholly withdrawn into the cuticular pores. This sinuous appearance is often seen in alcoholic material, and may be accounted for in two ways: the gradual stupefaction of the worm in alcohol may cause a contraction of separate portions of the protoplasm instead of an even contraction of the whole cell, or there may be first an even contraction, and afterwards a relaxation of part of the protoplasm. When a sense-hair is drawn into its canal, the part which normally lies in this canal is below the cuticula. This part is then seen to be of the same finely granular protoplasm as the part of the cell below it, and it generally shortens and widens. This contraction of the sense-cells and withdrawal of their hairs into the pores in the cuticula may be a normal process in response to external irritation, but is probably an unnatural contraction caused by the alcohol. The more delicate nature of the sense-cells and their comparative freedom from contact with each other permit reagents to act on them to a greater extent than on the other epidermal cells.

In these haematoxylin preparations, bundles of lightly stained fibres are found passing through the circular muscle-layer and pressing against the basement-membrane of the epidermis. Each bundle is enclosed in a nucleated membrane and its

fibres can often be traced through the basement-membrane into the base of the epidermis, but it is impossible to discover their further course in these preparations. That these bundles of fibres are nerves can be proved by tracing them, in serial sections, to the central nervous system. To avoid repetition, a description of the course of these nerves will be left until the silver nitrate preparation is described.

In these preparations, the sense-organs have been found in the epidermis of every region of the body except in the clitellum, but they are most numerous in the anterior and posterior metameres. In longitudinal sections, these organs are found most often in the cephalic border and in the median zone of a metamere. In cross sections, the most noticeable ones are found in sections passing through the setae. The form of a cephalic border of a metamere — curving down as it does to the intersegmental groove — causes a cross section through this region to pass obliquely through the epidermis. Therefore the sense-organs in the cephalic border of a metamere are easily overlooked in such sections. The sense-organs in the metameres back of the clitellum differ from those described for the anterior metameres merely in size. As the epidermis is thinner in these metameres, the organs are smaller — usually about 45μ high and 16μ wide. In a few caudal metameres, the sense-organs again increase in size.

The sense-organs not only occur in the epidermis, but Miss Randolph found them among the epithelium cells lining the buccal cavity. So far as I know, they have not been found in this region before. The sense-organs are here found as far caudad as the median zone of the fourth segment, *i.e.*, almost to the caudal limit of the buccal cavity. In this entire tract the sense-organs are larger and more numerous in the dorsal than in the ventral epithelium. In a given section through the cephalic half of the buccal cavity, the number of sense-organs in the dorsal wall varies from 18 to 31, and in the ventral wall from 0 to 7. In the caudal half of the buccal cavity the same comparative difference exists, but the organs are fewer in number. This difference in the relative number of organs in the two walls may be due to a difference which exists in the form

of these walls. The dorsal wall curves evenly ventrad into the buccal cavity, while the ventral wall is much convoluted longitudinally. The sense-organs in the first part of the buccal cavity are shorter and of greater average width as compared with their height than those of the epidermis, about 76μ high and from 50 to 60μ wide ; toward the caudal end of this cavity they diminish in size, and become only 20μ high and 10μ wide. The sense-hairs in the first half of the buccal cavity are from 4 to 6μ long, over twice as long as those of the epidermal sense-organs. The cuticula over a sense-organ in the buccal cavity is no thinner than that covering the surrounding cells, and the summit of a sense-organ does not project above these cells. Sometimes the cuticula passes evenly over the sense-organ from the surrounding cells; more often, however, it is indented around the sense-organ so that the summit of the latter really projects into an elevated area of cuticula, but this area is not elevated above the general cuticular level. The base of the sense-organ is often rounded and then presses the basement-membrane, which is very thin under the epithelium, out among the muscle-fibres. The sense-cells of the organs in the cephalic part of the buccal cavity do not differ from those of the sense-organs in the epidermis. In the caudal part, they are comparatively wider and have a more rounded nucleus.

Even in the cephalic part, the sense-cells and also the epithelium cells seem less crowded, and the former have larger spaces between them than in the epidermis. This may be due to the absence of the gland-cells from the epithelium. In these clear spaces between the sense-cells one often sees delicate fibres. These are sometimes cut walls of cells, but usually prove to be the very slender bases of some sense-cells. It was suggested in the description of the sense-organs of the epidermis, that the varying height of the nuclei of the sense-cells was merely due to the necessity of these cells adapting themselves to the space they were in. This receives confirmation from the position of the nuclei of the sense-cells in the buccal cavity. In this region, where the sense-cells have more room, all nuclei are generally found below the middle height of the sense-organs (Pl. XIII, Fig. 2).

A Study of the Epidermis by Means of Golgi's Silver Nitrate Method.

Effect of the stain on epidermis in general. — In sections prepared by this process, the cuticula was usually stained black and a black precipitate deposited beneath it. This rendered difficult a study of that portion of the epidermis just beneath the cuticula. The supporting cells remained clear or were stained a uniform black; in the latter case their basal processes were readily seen. The gland-cells were never stained either wholly or in part, but they could be easily distinguished because of their shape and contents. The basal cells were never stained. Sometimes the basement-membrane was obscured by a thick deposit of silver, but it generally showed with perfect clearness as an unstained, apparently structureless layer between the epidermis and the circular muscle-layer.[1]

Intraepidermal nerve-fibres. — The most striking feature in these sections is the presence of more or less deeply stained fibres *between* the epidermal cells, and in no instance connected with the bases of these cells (Pl. XIII, Figs. 3, 5, and 6). That these fibres are nerve-fibres there can be no doubt. That these are really fibres and not the edges of lamellae formed by the deposit of silver in intercellular spaces may be shown by an examination of sections which pass obliquely through the epidermis. Between the cell walls are seen sections of these fibres appearing as black dots; as the course of the fibres is oblique to the surface of the section, they may be traced down by focusing, and seen to be really fibres. That these are nerve-fibres is shown by two facts; first, by the fact that they present precisely the same appearance as undoubted nerve-fibres which are found among the muscles and in the central nervous system — the appearance of a clear, glistening thread sur-

[1] In all my preparations the blood-vessels among the circular muscles show distinctly either stained or unstained. These blood-vessels press closely against the basement-membrane, forming here great loops and coils. Lenhossék ('92) stated that the blood-vessels in his preparations enter the epidermis itself. Although I have searched my preparations carefully, I have found no instance of this outside of the clitellum. Lenhossék has a later paper (Die intraepidermalen Blutgefässe in der Haut des Regenwurmes, in *Verhandl. Naturf. Gesell. Basel*, Bd. 10, Heft I, p. 84) which I have not seen.

rounded by a black deposit; secondly by the fact that they may be traced into the central nervous system.

Both cross and longitudinal sections show that these fibres pass between the epidermal cells from the basement-membrane almost or quite to the cuticula. Some of the fibres are simple, some branch one or more times. It is hard to decide definitely as to their ultimate endings. Some become very slender and seem to end freely between the cell walls at varying distances below the cuticula; most of the fibres pass almost to the cuticula, where their ends are lost in the black deposit which is usually found there. The fibres vary in diameter and depth of stain; they are sometimes straight and of uniform caliber, but usually a little sinuous and varicose. In a few cases the upper end was found to turn at right angles and to run for a short distance just beneath the cuticula, sometimes again turning and running a very short distance toward the basement-membrane. There is sometimes found an appearance which suggests an anastomosis of these fibres in the epidermis (Pl. XIII, Fig. 5). A careful study shows that, in such cases, the fibres merely cross one another.

Sometimes in the basement-membrane, sometimes among the cells of the basal layer of the epidermis, are larger and more deeply stained fibres. In sections which pass obliquely through the epidermis, it is easy to see that the fibres between the epidermal cells arise from these fibres which pass along the base of the epidermis. It is not difficult to find places in cross sections in which the same fact is clearly shown (Pl. XIII, Figs. 3 and 6). These epidermal fibres often appear, at first sight, to be connected with the basal processes of the supporting cells, but a careful study shows that in every such case the fibres really run under or over these processes. Sections passing obliquely or tangentially through the base of the epidermis show that these subepidermal fibres form a freely branching network (Pl. XIII, Figs. 8, 9). The fibres of this network often appear to anastomose, but careful focusing shows that in the greater number of such cases the fibres merely cross each other. In a smaller number of such cases there seems to be a real anastomosis (Pl. XIII, Fig. 9, *a*).

In cross and longitudinal sections, there appear bundles of stained fibres passing to the epidermis through the circular muscle-layer (Pl. XIII, Fig. 3). These fibres are often varicose and sinuous, and appear imbedded in or surrounded by an unstained substance. In sections in which the fibres show at the very base of the epidermis, it is easily seen that they pass through the basement-membrane and become continuous with the fibres of the subepidermal network. Hence the intraepidermal nerve-fibres are branches of fibres which approach the epidermis in the same position as the epidermal nerves described in the haematoxylin preparations. As will be seen presently, these fibres are efferent nerve-fibres. These intraepidermal nerve-fibres appeared in every preparation made, and have been found in all regions of the earthworm except in the clitellum, which has not been studied for this purpose; but they appear to be more numerous in the caudal metameres. The fibres have been found between the supporting cells, but appear to be more numerous in those regions in which the gland-cells are more abundant. As these fibres have not been obtained by any other stain, it is not known whether fibers have remained unstained in parts of the epidermis where they have not been seen. However, the great readiness with which these fibres took the stain would seem to oppose this idea. No intraepidermal fibres have been found in the sense-organs, and they are not numerous in their immediate vicinity. This latter fact is probably connected with the absence of gland-cells in the immediate vicinity of a sense-organ.

Between the cells of the epithelium lining the buccal cavity are nerve-fibres which are similar to the intraepidermal fibres (Pl. XIII, Fig. 10). The epithelium cells and the cuticula covering them is never stained, consequently the endings of these fibres can be seen more readily than in the epidermis (Pl. XIII, Fig. 7). Some fork once; many end in what appears to be a flat plate, but it is impossible to state that this is anything different from the "artefacta" often seen in the course of a fibre. These fibres in the buccal epithelium may be traced to the cephalic ganglia or aesophageal ring.

The greater abundance of the intraepidermal fibres in regions

in which the gland-cells are more abundant suggests that the function of these nerve-fibres may be to control the secretion of the gland-cells. But the fact that the fibres are also found among the supporting cells and in the buccal epithelium indicates that, even if the above suggestion be true, this is not their only function. The intraepidermal fibres may have for their function the control of the general metabolism of the epidermal cells, or it may prove that their function is sensory.

Sense-organs. — Although no difficulty has been experienced in staining the intraepidermal nerve-fibres, there has been an uncertainty in the action of the stain on the sense-organs. No difficulty has been found in recognizing the latter, whether stained or unstained, in all preparations in which they occur. In sense-organs which are unstained, even in cases in which it is impossible to distinguish the separate cells, the organs themselves may be easily identified by the elevation of the cuticula above them and by the layer of deeply stained covering cells around them (Pl. XIII, Fig. 3). In many of the sense-organs the sense-hairs, sometimes stained, sometimes clear, could be seen projecting from the cuticular elevation, thus affording an unmistakable evidence of the presence of the sense-organs. When the sense-cells of the sense-organs are stained (Pl. XIII, Figs. 11–14), the covering cells are sometimes stained, sometimes unstained. In all cases the basal cells of the sense-organs, as well as those in the epidermis, are unstained. The sense-cells themselves have in my preparations taken the stain so differently from any other cell of the epidermis that they could be easily distinguished even under a low power. The covering cells of the sense-organ and the supporting cells of the epidermis stain black or a blackish brown, and no trace of a nucleus can be seen in them. The sense-cells stain a reddish brown, and usually the nucleus shows as a clear oval spot. This difference between the color taken by the supporting cells and that taken by the sense-cells must be due to some intrinsic difference between them; the nerve-fibres of the sense-cells have the same brown color, instead of staining black like the other nerve-fibres. Since these sections were prepared, the sense-cells have darkened a little, and in some cases the nuclei have

become indistinct. Not only are the supporting cells more nearly black, but their walls have an uneven appearance, and a cross section shows that the silver is deposited on them irregularly and rather thickly. The sense-cells present a smooth appearance, and look as if their walls were really stained. But cross sections of these cells show a delicate unstained wall, which has the same clear, glistening appearance noticed in the nerve-fibre, and, on the outside, a very thin, evenly applied deposit of the silver.

Comparatively few of the sense-cells in a given organ are stained. But a study of a thin section under the oil immersion shows plainly the outline and nuclei of the unstained cells (Pl. XIII, Figs. 11–13). All the sense-cells, both stained and unstained, present a plump, rounded form, and taper to both ends from an enlarged part in which the nucleus lies. In short, they are of the same form as the sense-cells described in the sense-organs stained by haematoxylin, and are clearly the same thing. The summits of the sense-cells cannot be traced through the cuticula; the slender, converging peripheral ends of the cells blend into a dark mass, which is continuous with the blackened cuticula. It is impossible to trace a single cell through this mass, but a careful study of the summits of the sense-organs almost always reveals a group of short black or colorless hairs projecting from its surface. The summits of the sense-organs in which the cells were stained are not so elevated as in any other preparations; they appear as if there had been an unusual contraction of the sense-cells. Other facts support this interpretation. The sense-hairs do not project above the cuticula as great a distance as in the unstained organs, and the cells themselves appear more thickened.

The silver nitrate stain permits the character of the bases of the sense-cells to be clearly seen. The cells which have a nucleus near the cuticula extend toward the base of the sense-organ into a slender fibre as in the haematoxylin preparations, and this fibre is a nerve-fibre (Pl. XIII, Fig. 15, *a* and *b*). Those cells which have their nucleus at the center or near the base of the sense-organs sometimes end abruptly at the base (Pl. XIII, Fig. 15, *g*). In this case a nerve-fibre is attached

to the cell near the circumference of the base. Some of these cells simply taper into a mere fibre (Pl. XIII, Fig. 15, *b*). The larger number of such cells have a base which forks into two or more basal processes (Pl. XIII, Fig. 15, *c*, *d*, *n*, *m*). These processes, which are usually varicose, descend in an irregular course to the basement-membrane. In most cases they end at the basement-membrane, but sometimes they extend along the outer surface of this membrane for a short distance beneath the neighboring cells. One of these processes, and only one, is always produced into a nerve-fibre (Pl. XIII, Fig. 5, 11, 12). In cross sections it is often impossible to establish the connection of some of the cells with nerve-fibres or to trace the course of these fibres. In sections which pass obliquely through the epidermis the connection of each cell with a nerve-fibre and the course of these fibres along the base of the epidermis can be clearly seen.

Since but few of the sense-cells in a given section of an organ are stained — frequently only one or two — it sometimes appears as if these stained cells were not grouped into sense-organs. But a study of such cases under the oil immersion always reveals the fact that the cell in question is one of the sense-cells of a sense-organ. In every case I have been able to make out that the stained cell is but one of a group of cells of similar shape, the rest remaining unstained, and that this group is enclosed in covering cells which reveal the oval outline of the sense-organ. In almost every case a more or less marked elevation shows the summit of the sense-organ, and this elevation usually bears a cluster of short sense-hairs. All these facts clearly prove that every sense-cell in my preparations which might be taken at first sight for an isolated cell is but *one of the sense-cells of a sense-organ.* The sense-cells of some of the sense-organs at the entrance to the buccal cavity were found to be stained. These cells were like those of the epidermal sense-organs, except that they were usually more slender. The sense-organs of the buccal cavity itself were unstained and the characteristic shape of their cells could not be made out; but the presence of the organ itself could usually be detected by the convergence of the summits of the cells (Pl. XIII, Fig. 10).

The nerve-fibres proceeding from the sense-cells are readily distinguishable from those which supply the epidermis. They are smaller in diameter and consequently of more delicate appearance; in their course along the base of the epidermis they neither branch nor anastomose, but pass directly to the

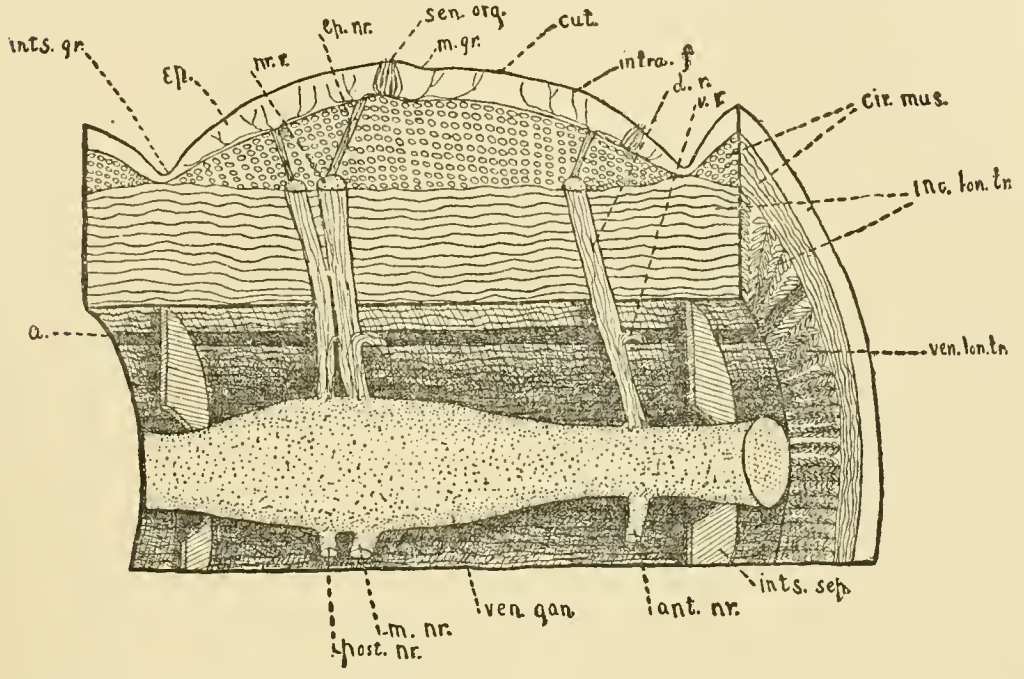

FIG. 1. A diagram showing a ventral ganglion and the course of its three pairs of nerves. In this diagram the ventral ganglion is represented in place and the body wall cut through on one side to show the nerves. *ant. nr.*, anterior nerve-trunk; *a*, space into which the ventral rami pass (this space is between the ventral longitudinal muscle-tract and the inner intersetal tract; *cir. mus.*, circular muscle-layer; *cut.*, cuticula; *d. r.*, dorsal ramus of nerve; *dor. lon. tr.*, dorsal longitudinal muscle-tract; *ep.*, epidermis; *ep. nr.*, epidermal nerve; *int. lon. tr.*, inner intersetal tract of longitudinal muscles; *int. long. tr. 2*, outer intersetal tract; *intra. f.*, intraepidermal nerve-fibres; *ints. gr.*, intersegmental groove; *ints. sep.*, intersegmental septum; *lat. lon. tr.*, lateral tract of longitudinal muscles; *m. gr.*, median groove around metamere, *m. nr.*, median nerve-trunk (anterior nerve of so-called "double root"); *nr. r.*, nerve-ring; *sen. org.*, sense-organ; *sub. n.*, subepidermal network; *ven. gan.*, ventral ganglion; *ven. lon. tr.*, ventral longitudinal tract; *v. r.*, ventral ramus of nerve.

nearest one of the epidermal nerves which traverse the circular muscle-layer; the sensory fibres from sense-organs on one side of a metamere always enter an epidermal nerve of that side; they never cross the mid-dorsal or mid-ventral line. In these nerves, the fibres from the sense-cells are never so deeply stained as the efferent nerve-fibres, — they usually present a clear brownish appearance. They are further distinguishable from the fact that they keep more nearly parallel to one another and run in uniformly sinuous lines.

Course of the nerves from the central nervous system to the epidermis. — As is well known, from each side of each ganglion of the ventral nerve-chain three great nerves take origin. The anterior pair of nerves arises just caudad of the anterior septum of the metamere. The middle and posterior pairs, which lie so closely together that they are often called "double nerve-roots," arise just caudad of the middle of the ganglion

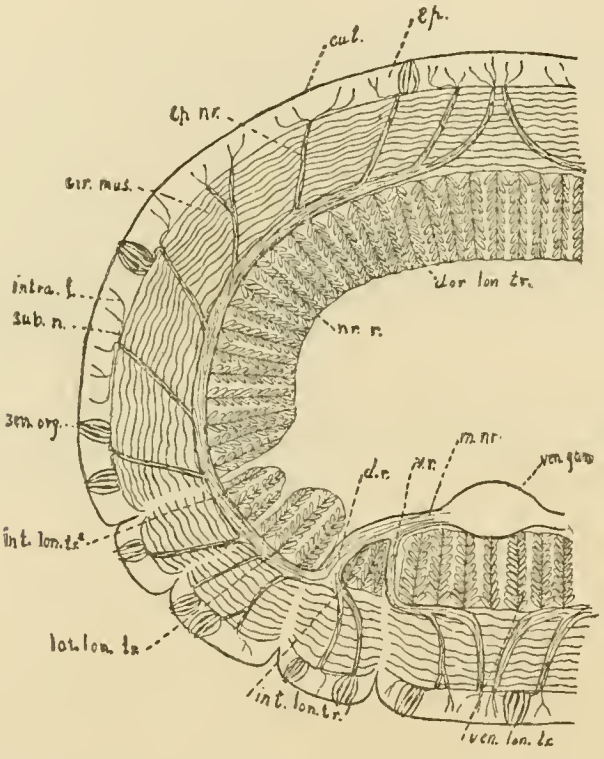

Fig. 2. A diagram showing the course of the median nerve-trunk and its formation of a nerve-ring. This diagram holds good for the other nerve-trunks, except that the sense-organs would not be so numerous as in this. For reference letters, see text, Fig. 1.

(see text, Fig. 1). All of these nerves pass latero-ventrad to the inner surface of the longitudinal muscle-layer, and there divide into a ventral and a dorsal ramus (see text, Fig. 2). These rami pass to the inner surface of the circular muscle-layer, the ventral ramus using the space between the ventral and inner intersetal tract of longitudinal muscles, the dorsal ramus using the space between the inner intersetal and lateral tracts. On reaching the circular muscle-fibres, the dorsal ramus sends a very short branch ventrad between the circular and longitudinal muscle-layers, and then turns and passes dorsad and slightly caudad between the two muscle-layers. As the dorsal ramus

approaches the mid-dorsal line, it leaves the space between the two muscle-layers, runs among the circular muscle-fibres, and finally reaches the base of the epidermis near the mid-dorsal line. The dorsal ramus of one side never crosses the mid-dorsal line to the other side of the metamere. The ventral ramus of each nerve-root turns between the two muscle-layers and passes to a point near the mid-ventral line, but never crosses this line (text, Fig. 2). There are thus formed in each segment three nerve-rings, which are incomplete in the mid-dorsal and mid-ventral line and which lie for the greater part of their course between the circular and longitudinal muscle-layers. Each of these nerve-rings is formed of four parts, the two dorsal and the two ventral rami of a pair of nerves. Back of the anterior metameres from which the diagram for text, Fig. 2, was made, an "accessory" tract of longitudinal muscles appears between the ventral tract and the inner intersetal tract. Each of the three nerve-roots then passes directly to the circular muscle-fibres through the space between this accessory and the ventral tract, and does not divide into its dorsal and ventral rami until the circular muscles are reached. In this case no ventral branch is given off from the dorsal ramus, and each nerve-ring appears more complete in that each half can be traced directly from the mid-dorsal around to the mid-ventral line, and is not interrupted in the region of the inner intersetal tract, as shown in the diagram. From each of these nerve-rings, the epidermal nerves before noted pass to the epidermis, and as they are given off the dorsal and the ventral rami of the nerve-rings become smaller. The epidermal nerves leave the nerve-rings at various angles but are generally inclined cephalad ; they rarely approach the epidermis directly at the base of a sense-organ, but they are most numerous in those regions in which the sense-organs are most numerous. The epidermal nerves from the anterior nerve-ring supply the epidermis of the anterior part, probably almost half, of a metamere ; those from the middle nerve-ring supply the epidermis in the zone which includes the setae, and those from the posterior ring supply the posterior part of the metamere.

From the ventral nerve-chain, nerve-fibres, apparently arising from the small ganglion-cells, pass out by each of the nerve-roots into the three nerve-rings. A careful study has not been made of the nerve-fibres going to the muscles, but I have observed that they may arise from any part of a nerve-ring. Other fibres which cannot be told from the nerve-fibres of the muscles pass from the nerve-ring through the epidermal nerves and form the subepidermal network previously described. In no case have these efferent fibres been seen to be connected with any cell in their course from the ventral ganglion to their ending in the epidermis. The sensory fibres pass without branching, anastomosing, or changing in diameter, to the central nervous system, which they reach by the same course followed by the efferent fibres in passing to the epidermis. Some of the epidermal nerves contain only efferent fibers, some contain both efferent and sensory ; some have been noticed which contain more sensory than efferent fibres, but it is rare to find one which contains only sensory fibres. Each nerve-ring and each nerve-trunk contains both the sensory fibres and the efferent fibres passing to the epidermis. Since the sense-organs are less numerous in the posterior half of a metamere, the posterior ring and its corresponding trunk contains a smaller number of sensory fibres than the others. Since the sense-organs are usually larger and more numerous around the middle of each metamere, the middle nerve-ring and its trunk contain more sensory fibres than the others.

In the central nervous system, each sensory fibre divides into two branches. One branch passes caudad, one cephalad, into the ganglia of the next metameres. Each branch ends freely. Of course it is impossible to trace an individual fibre from its origin in a sense-cell to its ending in the central nervous system, but these sensory fibres can be so readily distinguished from all other fibres that it is easy to identify them in the great nerve-trunks and in the ventral ganglia. The only cells with which these sensory fibres are connected are the sense-cells of the epidermal sense-organs.

Distribution of the Sense-Organs.

In a surface view of the cuticula the two layers of fibres which form it, the comparatively large openings of the gland-cells, the nephridial openings, the cuticular sacs of the setae, and the intersegmental grooves may be readily identified (Pl. XIII, Fig. 4, and Pl. XIV). Attention is usually first directed to the cuticular elevations over the sense-organs by the absence of the gland-pores over a little area around each elevation. These cuticular elevations themselves appear as irregularly rounded raised spots, which contain numerous openings smaller than the pores of the gland-cells (Pl. XIII, Fig. 4). Each one of these openings is the outer end of one of the pore-canals seen in sections, and, normally, a sense-hair protrudes through each. Each opening lies at the intersection of two short straight lines, each of which is a line of contact of two adjacent cuticular fibres. The existence of these two intersecting lines shows that both layers of the cuticular fibres are present over the sense-organs. The pore-canals through which the sense-hairs pass are thus spaces left between the fibres of the cuticular layers at their intersection. That is, the pore-canals are such openings as might be made by taking a layer of fibres, laying a second layer over and at right angles to the first, and then forcing a blunt instrument through both layers between their threads. Whether these openings always exist between the cuticular fibres, or are made by an outward growth of the sense-hairs, has not been determined. Even if they always exist, the presence of the cross-like marking at each opening shows that the sense-hair pushes the fibres still farther apart. When the cuticula is removed, the sense-hairs are usually pulled out of these canals. Occasionally the hairs are torn from their cells and remain projecting above the cuticula. A study of the distribution of the cuticular spots over the sense-organs forms the most ready means of determining the distribution of the latter.

The cuticular spots are found on every metamere of the body, and appear on the clitellum metameres after the clitellum has disappeared, and their size and distribution shows the following facts:

1. The sense-organs are largest and most numerous in the prostomium and first metamere. Here they are irregularly distributed, so that there is no evidence of a distinct grouping.

2. Distinct groups or zones of sense-organs may be recognized over the rest of the body. It should be borne in mind that every metamere contains sense-organs irregularly scattered over it outside of these groups and zones. The latter are merely noticeable from the fact that the organs in them are larger or more numerous than elsewhere. In the metameres back of the first the following arrangements of the sense-organs may be noted (Pl. XIV):

(*a*) There are always more sense-organs in the cephalic than in the caudal half of a metamere. This is also true of the first metamere.

(*b*) In the fifth or sixth metamere, the organs are seen to be more numerous in a zone which passes around the cephalic margin of the metamere. Passing caudad, this *cephalic zone* increases in prominence for six or seven metameres. It then becomes reduced to a single irregular row of large sense-organs, and remains as such until near the caudal end of the worm. In the caudal metameres, it gradually loses its prominence by a decrease in size of its organs. In this cephalic zone the smallest organs are generally found on the dorsal surface, and the largest near the nephridial opening.

(*c*) In the second metamere, there appears a zone of somewhat larger organs, which occupies the elevated caudal border of a slight groove that always passes around each metamere just cephalad of its median circumference. This zone is in line with the setae, and may be called the *median zone*. In the third and fourth metameres this median zone is well marked. From this point on, it diminishes in width until, back of the clitellum, it is reduced to a single irregular row of large organs. Continuing caudad, the organs of the median zone are found to diminish in size until about the last seventh of the body is reached. They then increase in size to the caudal end of the body. As a rule the smallest organs in this median zone occupy its dorsal part, and the largest ones are near the setae.

(*d*) Around each nephridial opening, there is always a thick cluster of small organs which may be called the *nephridial group*. The sense-organs of this nephridial group are always found around the nephridial opening, *no matter what its position*. The nephridial group is more prominent at the cephalic end of the worm, and diminishes in size toward the caudal end.[1]

It will thus be seen that the sense-organs are most numerous and largest at the two extremities of the earth-worm; that the most prominent organs at these extremities are those of the median zone; that those most prominent in the middle region of the body are those of the cephalic zone; and that the degree of prominence of the cephalic zone in any one region is inversely proportional to that of the median zone. If the nephridial opening really had the definite position often assigned to it — in front of the outer one of the inner pair of setae — a distinct lateral line of larger organs might be traced along both sides of the body. But the variability in the position of the nephridial opening, not only from metamere to metamere, but also on opposite sides of the same metamere, accompanied as it is by a variability in the position of the nephridial group of sense-organs and the position of the largest organs of the cephalic zone, destroys all lateral symmetry in their distribution. The cuticula from the ventral surface of the prostomium and from the buccal cavity shows the cuticular markings of the sense-organs irregularly distributed in both places, and occurring in as great numbers on the ventral surface of the prostomium as on its dorsal surface. The cuticular spots from the buccal cavity are less distinct, owing to the fact that the cuticula in this region is not elevated over the summits of the sense-organs. A groove often surrounds the cuticular spot, the same groove noted in sections of the sense-organs from the buccal cavity.

In a worm about 19 cm. long, which contained 153 metameres, there was found to be an average of 1000 sense-organs

[1] It is difficult to explain the presence of this group around the external opening of a nephridium unless it serves as a guard against parasites. The cuticular spots are exactly like those found over the rest of a metamere, and sections show that the underlying sense-organs have the same structure as those found elsewhere.

to a metamere, making about 150,000 in the whole worm. In the specimen from which the chart was made, there were about 1900 sense-organs on the first metamere and the upper surface of the prostomium, 1200 on the tenth metamere, and 700 on the fifty-sixth. These numbers are, of course, only approximate, but they give some idea of the great abundance of these organs.

The various zones and groups of sense-organs may be readily seen with a hand lens in living specimens, unstained alcoholic material, and in those stained with haematoxylin, if a large worm is used for the examination. The sense-organs appear as small, slightly elevated spots which reflect the light more strongly than the surrounding surface. Those organs which occur in line with the setae are most readily found. In the study of living specimens it is interesting to note that, when disturbed, the worm often draws the entire prostomium into the opening of the buccal cavity, thus protecting the numerous sense-organs on the prostomium as well as closing the opening. During regular contraction of the earthworm, the cephalic and caudal metameres, especially the former, are very much elevated around the median part, thus bringing the median zone into great prominence, while the border of adjacent metameres are so closely pressed together that the cephalic zone is concealed. This may account for the prominence of the median zone at the two ends of the worm. Further support for this interpretation may be found in the fact that in the metameres of the middle region of the body, where the cephalic zone is prominent, there is much less contraction, and consequently the cephalic zone is not apt to be concealed.

If a transparent worm about 15 mm. long be examined in a watch-glass of water under a cover-glass with a 4 mm. objective and 4 ocular, the sense-organs may be demonstrated. As the worm moves about in the little space thus formed, it is possible to observe continuously particular spots on the surface. The clusters of sense-hairs may then be distinctly seen radiating outward from the rounded elevation of the cuticula over the sense-organs.

Summary.

The foregoing observations seem to me to warrant the following statements of facts:

1. The epidermis, exclusive of the sense-organs, contains three kinds of cells, arranged in two layers: an outer layer of gland-cells and supporting cells, and an inner layer of small basal cells.

2. The supporting cells and gland-cells are not connected by intermediate forms, but both are connected by intermediate forms with the basal cells.

3. The epidermis is covered exteriorly by a cuticula composed of at least two layers of fibres.

4. The epidermis is separated from the circular muscles by a basement-membrane.

5. There are nerve-fibres ending freely between the cells of the epidermis; these fibres are more numerous among the gland-cells.

6. These intraepidermal fibres arise as efferent fibres in the central nervous system, and those in each half of a metamere come from the corresponding half of a ventral ganglion, or cephalic ganglion and aesophageal ring if in the prostomium and first metamere.

7. The efferent nerve-fibres leave the ventral ganglia by each of the three great paired nerves which arise from a ganglion, and reach the epidermis by the regular course of these nerves.

8. Each of these nerves passes through the longitudinal muscles to the inner surface of the circular muscle-layer and divides into a dorsal and a ventral ramus.

9. These rami pass between the two muscle-layers to the mid-dorsal and mid-ventral line. The rami of each pair of nerves thus form a nerve-ring which is incomplete dorsally and ventrally, and three of these rings thus exist in each metamere.

10. From these three nerve-rings, epidermal nerves pass through the circular muscle-layer to the epidermis.

11. The efferent nerve-fibres of these epidermal nerves pass through the basement-membrane and form a subepidermal network.

12. From this network, the intraepidermal nerve-fibres pass between the cells of the epidermis.

13. In the epidermis are sense-organs, each of which is composed of a group of sense-cells covered by a layer of modified supporting cells. In the base of each organ are usually a few small basal cells.

14. These sense-organs are also present in the epithelium lining the buccal cavity.

15. The sense-cells taper toward both ends; the outer end of each cell extends into a fine hair which passes through a pore-canal in the cuticula above it and projects beyond the latter; the basal end of each sense-cell is always produced into a sensory nerve-fibre.

16. The sense-cells are the only cells with which the sensory fibres are continuous; they are therefore ganglion-cells.

17. The sensory nerve-fibres pass along the basement-membrane to the nearest epidermal nerve. These fibres then take the same path to the central nervous system that the intraepidermal fibres took coming from it, and they enter a ventral ganglion by each of the three nerve-roots on their own side of the metamere.

18. Most epidermal nerves, each nerve-ring, and each nerve-trunk thus contain both efferent and afferent (sensory) nerve-fibres.

19. In the ventral ganglia each sensory fibre divides into two branches; one passes caudad, one cephalad; each ends freely in the next ganglion.

20. The sense-organs are distributed over the entire surface of the body, but are most numerous and largest at each end.

21. In most metameres a cephalic and a median zone of larger organs may be distinguished.

22. A nephridial group of small organs occurs around each nephridial pore.

In view of the conflicting accounts of the sensory structures in the epidermis of Lumbricus, I have thought it best to

restate the facts in the above form. All the facts in the foregoing description which have been described by previous investigators have been carefully verified in my own work. In the following critical summary of the literature, I have tried to indicate briefly the facts previously described and also those not before noted.

Summary of the Literature on the Sensory Structures in the Epidermis of Lumbricus.

The early observers, such as Pontallie ('53), Clark ('57), and Lankester ('65), considered that the upper lip of Lumbricus served as a sense-organ because of its rich nerve supply and its mobility. After the cellular structure of the epidermis had been demonstrated, investigators began to seek an explanation of the well known sensitiveness of Lumbricus by referring it to definite cellular elements of the epidermis. This cellular structure was first perceived by Leydig ('65), who discovered the supporting cells, gland-cells, and sense-organs.

Gland and supporting cells. — Leydig ('65) considered the gland-cells to be sense-organs having the appearance of unicellular glands, because, when isolated, he found an apparent nerve-fibre connected with each. Below the supporting cells of the upper lip, he described and figured, "verästigter Wurzelnerv," which he considered a prolongation from the bases of these cells. Perrier ('74) supported Leydig's observations, although he stated that positive proof was needed as to the character of these apparent nerve-fibres. Mojsisovics ('77) found the same network below the supporting cells. Vejdovský ('84) considered that these apparent nerve-fibres might be either connective tissue fibre or efferent nerve-fibres passing to the epidermis as to any other organ, and that their presence was no indication of the sensory nature of the common elements of the epidermis. Ude ('86) considered Leydig's observations on the basal network of the supporting cells probably correct. It seems to me very probable that the fibres attached to the gland-cells were really nerve-fibres, the intraepidermal fibres described in this paper ; these might easily come away with the isolated

gland-cells. The network from the bases of the supporting cells was also probably bits of these nerve-fibres apparently continuous with the true basal processes of these cells. Leydig's figures of these cells show that they are supporting cells, and precludes the possibility of referring this to the true sense-cells. The basal processes of the supporting cells are understood when one remembers the layer of basal cells beneath them. Ude ('86) and Cerfontaine ('90) are the only ones who refer to these basal cells. There is no connection of nerve-fibres with the bases of either gland or supporting cells.

Sense-organs. — Leydig ('65), the discoverer of the sense-organs, saw them as "blase rundliche Flecken," which appeared by focusing below the surface. He described these spots as five or six times larger than the surrounding cells, and often limited by pigment. Of their minute structure he could only see that each was formed by a certain grouping of cells. I have found only the sense-organs of the dorsal surface of the head end limited by pigment. This may be of physiological interest. Perrier ('73) recorded his inability to find any sense-organs in Lumbricus, but he made only surface examination. Mojsisovics ('77) was the first to describe the sense-organs as seen in sections, and to figure their cellular structure. His figures do not show the true form of the sense-cells. He represented these cells as differing from the supporting cells merely in the possession of terminal hairs. He gave F. E. Schulze credit for discovery of the "Porencanälchen," through which the sense-hairs pass, but he himself seems to have first seen the corresponding cuticular markings in surface view. Darwin ('82) referred to Lumbricus as "remarkably deficient in the several sense-organs." Vejdovský ('84) and Ude ('86) verified Mojsisovics' description of the sense-organs and their cuticular markings, but Ude failed to find the sense-hairs. It seems to me probable that in his preparations these hairs were drawn into the pore canals. Vogt and Jung ('88) were not only unable to find any sense-organs themselves, but stated that such organs are unknown. Kulagin ('90) found the "Zellenanhäufungen" described by Ude. He probably refers to the sense-organs. Cerfontaine ('90), although he gave a more complete

description and illustration of the sense-organs of Lumbricus than any of his predecessors, added little to our knowledge of their structure. He likened the mode of arrangement of the cells in a sense-organ to the overlapping scales in an onion. A section through the outer surface of a sense-organ, consequently through the covering cells, does often give this appearance, but a good section through the center of an organ shows that there is no regularity in the arrangement of its cells. Cerfontaine gave the first satisfactory figure of the cuticular markings. Lenhossék ('92) and Retzius ('92, a and b) both denied the existence of definite sense-organs. The titles of some of the papers which contain descriptions of these organs appear in the footnotes to Lenhossék's article, but these descriptions are not referred to in his paper. It seems to me that no observer has correctly described the cells of the sense-organs.[1]

[1] An article entitled "Zur vergleichenden Anatomie der Oligochaeten" by Dr. Richard Hesse has recently appeared in the *Zeit. f. wissen. Zoologie*, — Bd. 58, p. 394, 1894, — in which the writer correctly describes the sense-cells and also arrives at the same conclusion concerning the nerve-cells of Lenhossék as I have presented. Hesse worked on several species of Lumbricidae, one of which, Lumbricus herculeus, Sav., is the same as that upon which I worked. Our results on this species are in general confirmatory; they differ in the following points: (1) Hesse finds in a sense-organ supporting as well as sense-cells, but does not note the small basal cells. He describes these supporting cells as of the same width throughout. I have looked through my preparations again, tracing single sense-organs through all their sections, and I feel warranted in believing that such supporting cells do not regularly form a part of a sense-organ. I have found, in organ after organ, nothing but cells which *taper to both ends* from the enlarged part in which the nucleus lies, and which end in a hair-like process passing through the cuticula. Moreover, I have found that the number of sense-hairs seen in a given section of a sense-organ corresponds to the number of cells in that section; and the number of pores over a sense-organ — as shown in the cuticular markings — corresponds to the number of cells usually present in a sense-organ of the same region. Sections which give cross-sections of sense-organs show that these organs often have one side deeply indented. In such cases, a longitudinal section of the same organ on this side would have shown some of the *covering* cells between the sense-cells, and these covering cells would appear like the supporting cells described and figured by Hesse. I would therefore consider that, if a sense-organ contains any supporting cells, these do not differ in form or peripheral ending from the sense-cells. (2) Hesse believes that the sense-organs in a given metamere "auf drei Gürteln liegen, die um das Segment herumlaufen." Of these his "mittlere Gürtel" must correspond to my median zone, while his "vordere" and "hintere Gürtel" must be composed of some of the small organs irregularly dis-

Isolated nerve-cells. — Kulagin ('88) described in the epidermis of several species of Lumbricus isolated sense-cells with a hair projecting through the cuticula and a base connected with a nerve fibre. As he gave no figures, and apparently did not use a specific nerve-stain, it is impossible to decide on the character of these cells. Lenhossék ('92), believed the sensitiveness of Lumbricus to be due to isolated nerve-cells which were scattered "an allen Stellen der Körperoberfläche mit Ausnahme der intersegmentalen Furchen." He stated that these cells "finden sich weder auf gewisse Gegenden beschränkt, noch an bestimmten Stellen zu besonderen Sinnesorganen angehäuft, sondern erscheinen über alle Gebiete der Epidermis gleichmässig vertheilt." I have been unable to discover any isolated nerve-cells. I have found many cells which

tributed over a metamere. His "vordere Gürtel" is not near enough to the intersegmental groove to correspond to my "cephalic zone." The different results we have arrived at in regard to the distribution and numbers of the sense-organs is due to the fact that my study was made by means of the cuticular spots and his by means of sections. In the latter the cephalic zone, the nephridial group, and many of the smaller organs are apt to be overlooked. (3) Hesse believes that the two ventral rami of a nerve-ring meet in the mid-ventral line. I have never found this to be the case. He found that the dorsal rami of the anterior and posterior nerve-rings always remained between the two muscle-layers. I have found that all three rings pass into the circular muscle-layer during the last part of their course dorsally. (4) Hesse describes groups of ganglion-cells in the course of the nerves of the prostomium. In discussing their function, he considers it probable "dass die sensiblen Fasern die von den Sinneszellen aufgenommenen Reize an die Ganglienzellen übermitteln, deren motorische Fortsätze an die Rückziehmuskeln der Oberlippe führen und deren Zusammenziehung veranlassen," but he has evidently not been able to trace these fibres. I have been able to find ganglion-cells which seem to be the same as those which he describes, but it does not seem to me that the fibres from the sense-organs end among these cells. In the course of the nerve-rings, Hesse has "nur einmal eine solche beobachtet, und zwar eine bipolar." My haematoxylin and silver nitrate preparation have not shown such ganglion-cells in the nerve-rings, but some alum carmine mounts which I have lately examined have revealed them in considerable numbers. I counted the number of these ganglion-cells in one half of each nerve-ring in the first thirteen metameres of one worm. The second metamere had but one on its median ring; the third had one on its anterior ring and four on its posterior ring. From the third through the thirteenth, the ganglion-cells occurred in every nerve-ring in numbers varying from two to eight. I could see that these cells were bipolar, and in several cases that a fibre started both towards and away from the central nervous system. I expect to continue my work on these ganglion-cells and to give more concerning them in the future.

correspond to Lenhossék's descriptions and illustrations of the nerve-cells, but I have found them to be *in every case* in one of the sense-organs seen by previous observers. It is my opinion that the isolated nerve-cells described by Lenhossék are the sense-cells of the sense-organs. It will be noticed that his diagram of the appearance of these cells in the epidermis (see Lenhossék, '92, Taf. V, Fig. 6) represents a section through the region of the setae, the very region in which the sense-organs are most numerous, and that he more often figures these cells in groups of two or three than isolated. Lenhossék's illustrations and descriptions of these "Nervenzellen" correspond exactly to the sense-cells of the sense-organs as they appear in my silver nitrate sections, except that I have never found the basal processes of these cells running as far along the base of the epidermis as he figures them. It seems to me not unlikely that some of the long ends of these processes may be parts of the subepidermal network. That Lenhossék failed to recognize these organs may be due to the fact that the silver stains but few of the cells of one organ. And if he used thick sections mounted without a cover-glass, it would probably be impossible to perceive the unstained sense-cells and the outline of the sense-organ.

Lenhossék figures his nerve-cells as ending at the cuticula. The difficulty of retaining the cuticula in position and the heavy deposit of silver made along it, seems to me to explain his failure to find the sense-hairs. In all my sections in which the sense-cells were stained, the sense-hairs were almost or quite withdrawn, and might therefore be readily overlooked. My own experience has shown me that a knowledge of the structure of the sense-organ as shown in haematoxylin preparations is necessary for a correct interpretation of the appearance obtained by the silver nitrate method. Lenhossék stated that in haematoxylin preparations his nerve-cells could scarcely be told from the supporting cells; I have found that the sense-cells of the sense-organs may be readily distinguished from the supporting cells in all my preparations.

Retzius ('92a) figures and describes the isolated nerve-cells of Lenhossék. He figures at the base of the epidermis a net-

work formed from the basal processes of these nerve-cells ; it seems to me probable that much of this network is really a part of the subepidermal network of the efferent fibres. Of the nerve-cells themselves, he says, "Eine Gruppirung derselben zu dicht gedrängten Gruppen oder Organen, wie von vornherein angenommen werden konnte, scheint nicht vorzukommen." In view of my interpretation of the facts, one statement which Retzius makes concerning an appearance in the cuticula is interesting. He describes, in cross-sections, an appearance of fine lines crossing the cuticula perpendicularly and then says, "an den Stellen wo die Sinnesnervenzellen die Cuticula berühren, sah ich ferner oft eine kleine hügelartige Erhöhung der letzteren und ihre lineäre Zeichnung verstärkt, so dass das Ganze den Eindruck feiner Stiftchen (oder Kanälchen) machte, ohne dass ich die Natur dieser Bildung sicher zu eruiren vermochte." From this description, I should judge that Retzius saw, at least in some cases, the cuticular elevation over a sense-organ and the fine pores piercing it. In the two illustrations which he gives of this appearance (*l.c.* Pl. VI, Fig. 2) the cuticula appears exactly like that over a sense-organ but the cell beneath seems to me to be a very large gland-cell.

Retzius found in the "Mundepithel" what he considered might be "Geschmackszellen." These were apparently fibres which passed through the epidermis and ended in an enlarged part under the cuticula. His figures of these do not admit of their being the sense-cells of the sense-organs.

Nerve-supply of sense-organs. — Vejdovský ('84) and Ude ('86) both described a direct connection of nerve-fibres with the sense-organs, but neither gave any evidence of this connection or any evidence that the fibres were really nerve-fibres. Lenhossék ('92) proved the direct connection of his nerve-cells with nerve-fibres. If his nerve-cells are the sense-cells of the sense-organs, he was the first to prove the connection of these organs with nerve-fibres. His account of this connection and his description of the course of these fibres has been with one exception confirmed by my own work; I have never found a sensory fibre crossing the circular muscles alone, but always

by way of the epidermal nerves. Retzius ('92) differs from Lenhossék in regard to the central endings of the sensory fibres. He found that these sometimes ended in the same ganglion which they entered, and that their ends were not always tapering, but were "in der Regel etwas knotig-varicös, ungefähr wie bei anderen sehr einfachen Nervenendigungen, gewöhnlich etwas gebogen und nicht selten etwas verzweigt." I have not found such endings. Retzius found in each half of the ventral nerve-cord three parallel longitudinal tracts of sensory fibres. Of these the outer and largest one received sensory fibres entering by all three nerve-roots; the median bundle was next in size and received fibres from the anterior and median nerve-roots; while the inner and most delicate tract received fibres only from the median nerve-root. That means that the sense-organs around the median zone of a metamere furnish fibres to all three tracts, those around the cephalic part to two, and those around the caudal part to but one. Apáthy ('92) believed that the sensory fibres described by Lenhossék and Retzius were not sensory but motor fibres, and were not connected with epidermal cells.

Distribution of the sense-organs. — Leydig ('65) found the sense-organs on both extremities of Lumbricus. Vejdovský ('84) extended the distribution over a few metameres back of the head; Ude ('86) extended it over the whole body, and first noted the median zone of sense-organs. Cerfontaine ('90) added to this the fact that this zone is situated on a crest of the epidermis. His statement that this crest is more prominent on the ventral surface has not been confirmed by my observations. No observer has noted the cephalic zone or the nephridial group, nor has any one attempted a systematic study of the distribution of the sense-organs. No notice has been found of the presence of the sense-organs in the buccal cavity.

Functions of sense-organs. — But little has been written on the functions of these sense-organs. Although Darwin ('82) did not know of their presence, his physiological experiments may aid in determining their function. He found in Lumbricus the sense of hearing lacking, the sense of smell feeble, the sense of taste well developed, the ability to perceive light

present on a few anterior metameres only, and the sense of touch highly developed over the whole body. Leydig ('65) seems to have regarded the sense-organs as "Geschmacksknospen." Vejdovský ('84) objected to this, and thought them "Tastorgane," an opinion which is shared by Ude ('86). Vogt and Jung ('88) stated that Lumbricus perceived light and *sound*. It is likely that they mistook the great sensitiveness of this worm to mechanical disturbance for an ability to perceive sound. Retzius, in writing of the probable function of the "Sinnesnervenzellen," says, "Der Regenwurm besitz keine anderen, höher entwickelten Sinnesorgane; er ist aber bekanntlich für Sinnesreize verschiedener Art empfindlich. Es wäre deshalb von ausserordentlich hohern biologischen Interesse zu erfahren, ob dieselben Sinnesnervenzellen diese verschiedenen Eindrücke vermitteln, oder ob einzelne derselben eine besondere physiologische Function haben." Since these sense-organs form the only known sensory apparatus of Lumbricus, and since their structure is not visibly different in different parts of the body, it is likely that they are sense-organs of a general nature capable of reacting to mechanical, chemical, thermal, or luminous stimuli. Those situated in the buccal cavity doubtless serve principally as organs of taste. The presence of a thick layer of pigment in the dorsal epidermis of the head metamere suggests an explanation of the fact that light is perceived only on the anterior metameres. Lenhossék homologized his nerve-cells with ganglion-cells of the dorsal roots of the spinal nerves.

Intraepidermal nerve-fibres. — Lenhossék ('92) brought up the question of intraepidermal nerve-endings but to decide against the possibility of their presence. After an examination of a large number of preparations, he stated, "kann ich nun das Vorkommen einer freien Nervenendigung in der Haut des Regenwurms mit grosser Wahrscheinlichkeit ausschliessen." As these intraepidermal nerve-fibres have appeared in every one of my sections, I can account for the fact that they were not seen by Lenhossék only on the assumption that the silver stain produced somewhat different results in his hands and in mine. That there was a difference in our results is

shown by the fact that the gland-cells were stained in his preparations while they were always unstained in mine, and by the fact that Lenhossék could not find a basement-membrane and did not notice the basal cells of the epidermis, while both of these structures showed in my preparations. Retzius ('92) says, "freie Nervenendigungen sah ich ebenso wenig wie v. Lenhossék im Hautepithel des Regenwurms." It is, however, evident that he saw such a nerve-fibre in the buccal cavity, but as he saw this appearance but once, judged himself mistaken.[1]

[1] After my work was completed and the account of it in the hands of the editor of this Journal, a preliminary paper by Dr. Alexis Smirnow, entitled "Ueber freie Nervenendigungen im Epithel des Regenwurms," appeared in *Anat. Anzeiger*, Bd. 9, No. 18 (June 23, 1894). In this paper Smirnow records his discovery of free-ending nerve-fibres in the epidermis of Lumbricus. My own discovery of these fibres was made in the spring of 1893, and briefly mentioned in a preliminary account of my work which was read before the American Morphological Society at the New Haven meeting in December, 1893.

In the main Smirnow's work on these free nerve-endings confirms my own; but his account differs from mine in the following particulars: —

1. Smirnow overlooks the presence of sense-organs and follows Lenhossék and Retzius in ascribing the sensitiveness of Lumbricus to isolated nerve-cells. These cells I regard as the constituent cells of epidermal sense-organs. I regard Smirnow's statement (*l.c.*, p. 574) concerning the striation and elevation of the cuticular over the "sensibel Nervenzellen" as strong evidence of this. He says: "Die Strichelung der Cuticula ist häufig schärfer ausgebildet an den Stellen, wo sich die äusseren Enden der Nervenzellen mit der Cuticula berühren, worauf bereits G. Retzius aufmerksam macht, ebenso wie auf die hügelartige Verdickung der Cuticula, die an diesen Stellen manchmal vorkommt. Ob diese Strichelung von einer Canalisation der Cuticula abhängt, kann ich ebensowenig entscheiden wie Prof. G. Retzius." My study has convinced me that these striations over the "Nervenzellen" are truly due to canals in the cuticula, and that the hairs borne by these cells pass through these canals to the exterior. The "hügelartige Verdickung" is, as I interpret it, the elevated summit of a sense-organ.

2. I have never found the protoplasmic processes of the "Nervenzellen" very long or forming a part of the "subepithelialen Plexus." In thick sections, bits of the subepidermal network sometimes appear as if continuous with these processes; in every case in which I have been able to trace the protoplasmic processes of the sense-cells, I have found that they end at or on the basement-membrane under the sense-organ in which the cell they come from is situated, and that their course is usually sinuous owing to the fact that they have to reach this membrane by passing between the small basal cells of the sense-organ, — cells which Smirnow does not mention.

3. I do not find that the sensory fibres form a part of the subepidermal network. I have always found that these fibres pass in bundles, without any connection with other structures in the epidermis, directly to the nearest epidermal nerve.

Conclusions.

From the foregoing study, the following conclusions seem to me to be warranted :

1. The epidermis of Lumbricus agricola, Hoffm. contains a

4. Smirnow believes that some of the intraepidermal nerve-fibres surround the sense-cells. Although I have searched carefully, I have failed to find any evidence of such connection between these fibres and the "Nervenzellen"—*i.e.*, the sense-cells of a sense-organ. In thick sections, it sometimes appeared as if the intraepidermal nerve-fibres penetrated the sense-organs, but under the oil immersion such fibres were found to be always outside of the sense-organs, among the supporting cells. I have preparations in which the intraepidermal fibres are stained in such numbers in the tissue between sense-organs that it seems to me unlikely that my failure to find fibres surrounding the sense-cells is due to a failure of the stain to act on such fibres.

5. Smirnow describes and figures the "Geschmackszellen" found by Retzius ('92) in the buccal cavity. Smirnow's figures and description of these so-called cells lead me to the conclusion that they are but the outer ends of ducts from the gland-cells which are so numerous in this region. He says: "Der verjüngte Teil der Zelle und der sich an ihn unmittelbar ausschliessende Fortsatz imprägniren sich viel schwächer, als der verdickte Teil der Zelle, wobei die Imprägnation mit dem Silbersalz an den Rändern des Fortsatzes intensiver ist, als in dem axialen Teile, der heller erscheint und zum Teil von schwärzlichen Krümeln und Brocken erfüllt ist. Unter diesen Bedingungen macht der Fortsatz den Eindruck eines hohlen Gebildes, eine Röhre, deren Lumen z. T. von krümligen Massen erfüllt ist. Dieser eigentümliche Bau lässt mich vorläufig zweifeln an der nervösen Natur dieser Gebilde." I have some sections stained by Kleinenberg's haematoxylin which were not treated with sodium bicarbonate and which have, therefore, faded. But the glands have retained the stain and stand out distinctly. I find, above the pharynx, glands from which long ducts pass in a sinuous course through the surrounding tissue to the upper wall of the pharynx. Each tube enters the epithelium of this wall and opens exteriorly by a minute pore in the cuticula. Just beneath the cuticula each duct is slightly enlarged. In fact, these tubes appear exactly like the illustrations which Retzius and Smirnow give of the so-called "Geschmackszellen." I find the ducts filled with minute, deeply stained particles of the secretion, which would answer to the "schwärzlichen Krümeln" seen by Smirnow; the secretion in the enlarged apex is often clear, homogeneous, which would explain the appearance which both Retzius and Smirnow thought might be a nucleus.

Smirnow's discovery was later verified by Retzius in an article entitled "Die Smirnow'schen freien Nervenendigungen im Epithel des Regenwurms," which appeared in the *Anat. Anzeiger*, Bd. 10, Nos. 3 and 4 (Oct. 6, 1894). In new preparations, Retzius succeeds in obtaining the intraepidermal nerve-fibres. He, himself, now feels doubtful concerning the nervous nature of the "Geschmackszellen" (or, as he calls them in this article, the "Kolbenfasern"). He finds these back of the "Mundhöhle," and says: "In eine Reihe von Präparates habe ich sie nun so massenhaft gefärbt gefunden, dass ich gestehen muss, das mir zuerst ebenfalls ihre nervöse Nature etwas zweifelhaft erschien."

sensory apparatus composed of definite groups of sense-cells whose outer ends pass through the cuticula as sense-hairs and whose inner ends give origin to nerve-fibres which pass directly to the central nervous system and there end freely.

2. The great number of these sense-organs, their existence over the entire body, their great abundance at the two extremities, and the zones of large ones in such positions as easily to be brought in contact with foreign bodies, accounts for the well known and extreme sensitiveness of Lumbricus.

3. These epidermal sense-organs were known to Leydig ('65), Schulze, Mojsisovics ('77), Vejdovsky ('84), Ude ('86), and Kulagin ('88), and their presence can be readily demonstrated.

4. As I have found no isolated nerve-cells in the epidermis of Lumbricus, I am able to account for those described by Lenhossék only on the assumption — which seems to me to be fully warranted by the facts — that he saw the sense-cells of the sense-organs, but failed to recognize the grouping of these cells into organs.

5. The sense-cells are the only cells with which these fibres are connected. They are therefore the nutrient centers of the sensory fibres and true ganglion cells.

6. If there is any differentiation in function between sense-organs of different regions, it is not correlated with any pronounced differences in structure.

7. The efferent nerve-fibres which pass from the central nervous system to the epidermis are not in continuity with any cellular element of the latter. They form a subepidermal network which gives rise to intraepidermal nerve-fibres which end freely between the epidermal cells.

Ann Arbor, Mich.,
June 18, 1894.

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REFERENCE LETTERS.

bas. c.	basal cells.
bas. m.	basement-membrane.
bl. v.	blood-vessels.
cir. mus.	circular muscle-layer.
cov. c.	covering cells.
cut.	cuticula.
cut. sp.	cuticular spot, surface view of elevation over sense-organ.
cut. sh.	cuticular sheath of setae.
ef. f.	efferent nerve-fibres going to epidermis.
epth.	epithelial cells lining buccal cavity.
gl. c.	gland-cell.
gl. p.	external opening of the gland-cell.
inters. gr.	intersegmental groove.
intra. f.	intraepidermal nerve-fibres.
met.	metamere.
mus. fib.	motor nerve-fibres of muscles.
neph. p.	nephridial pores.
p. can.	pore-canal for sense-hair.
pros.	prostomium.
sen. c.	sense-cell.
sen. f.	sensory nerve-fibres.
sen. h.	sense-hair.
sen. org.	sense-organ.
sup. c.	supporting cell.
sub. net.	subepidermal network.

DESCRIPTION OF PLATE XIII.

All the work was done with Zeiss apochromatic lenses. The figures were drawn to a scale of 9 mm. for .01 mm. and reduced one-half in the plate. Except where noted, they are from camera outlines with compens. ocular No. 8 and obj. 4 mm.; the details were filled in with the 2 mm. oil immersion. All the figures except 1, 2, and 4 are from silver nitrate preparations.

FIG. 1. A sense-organ from a cross section of an anterior metamere, haematoxylin preparation. The sense-hairs are retracted. In the circular muscle-layer is seen an epidermal nerve and a characteristic loop of a blood-vessel. It was impossible to distinguish all structures in lower right side of figure.

FIG. 2. A sense-organ from the buccal cavity, haem. prep. This shows the depression in the cuticula around the border of the summit of the sense-organ.

FIG. 3. Part of the epidermis from a cross section, showing the appearance of the sense-organs when unstained; the connection of the intraepidermal nerve-fibres with the subepidermal network and of this network with the efferent fibres is also shown. Some of the basal processes of supporting cells were made lighter to show the nerve-fibres passing across them.

FIG. 4. A bit of the removed cuticula showing the two layers of cuticular fibres, the gland-pores, and a cuticular spot over a sense-organ. The minute openings in the latter are the outer openings of the pore-canals of the sense-hairs.

FIGS. 5 and 6. Intraepidermal nerve-fibres among the gland-cells of a caudal metamere. The subepidermal network obscures the basement-membrane. In Fig. 6, the cuticula was absent and a heavy black deposit of silver took its place.

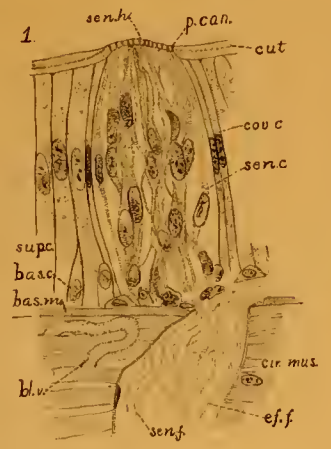
FIG. 7. Ends of intraepidermal nerve-fibres of the buccal cavity. Cuticula absent in *a* and *b*.

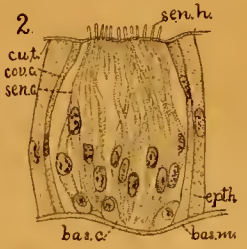
FIGS. 8 and 9. Surface view of the subepidermal network from sections which passed through the epidermis tangent to the basement-membrane. In Fig. 8 the fibres merely cross each other. In Fig. 9 they anastomose at the part marked *a*.

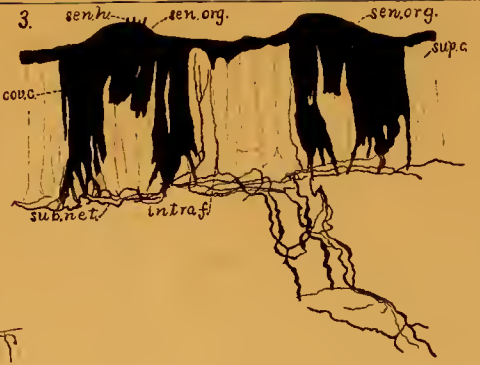
FIG. 10. Intraepidermal nerve-fibres and an unstained sense-organ in the epithelium lining the buccal cavity. The cuticula was absent and the basement-membrane could not be distinguished. Several efferent nerve-fibres and two blood-vessels traverse the circular muscles.

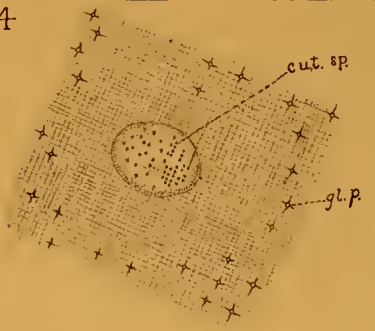
FIGS. 11–14. Sense-organs of the median zone from cross sections of an anterior metamere. The plump, rounded form and bright brown color of the sense-cells could not be represented in these illustrations. The section passed somewhat obliquely through the organs, so that the bases of some cells are cut off and the cuticula appears wider than is normal. A silver deposit beneath the cuticula adds to the apparent width of the latter. In Figs. 11 and 12 the connection of a sensory fibre with its sense-cell is shown.

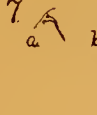
FIG. 15. Bases of sense-cells from oblique sections of the sense-organs of the ventral surface of the prostomium. The summits of the cells have been cut off.

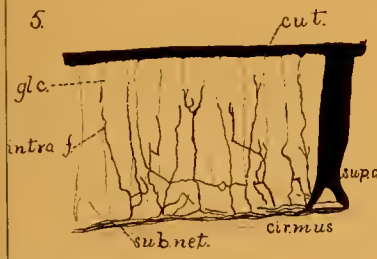


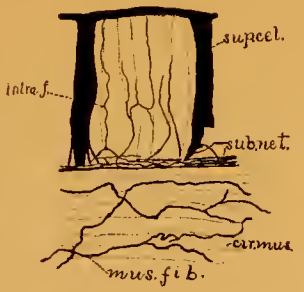


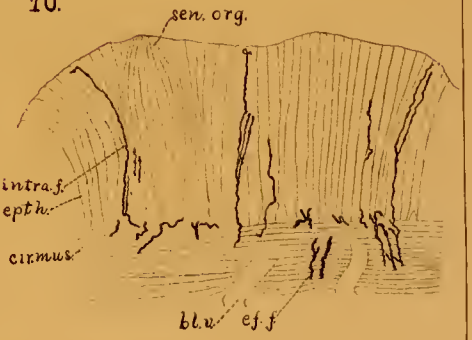


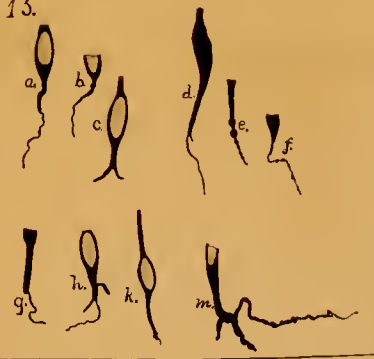








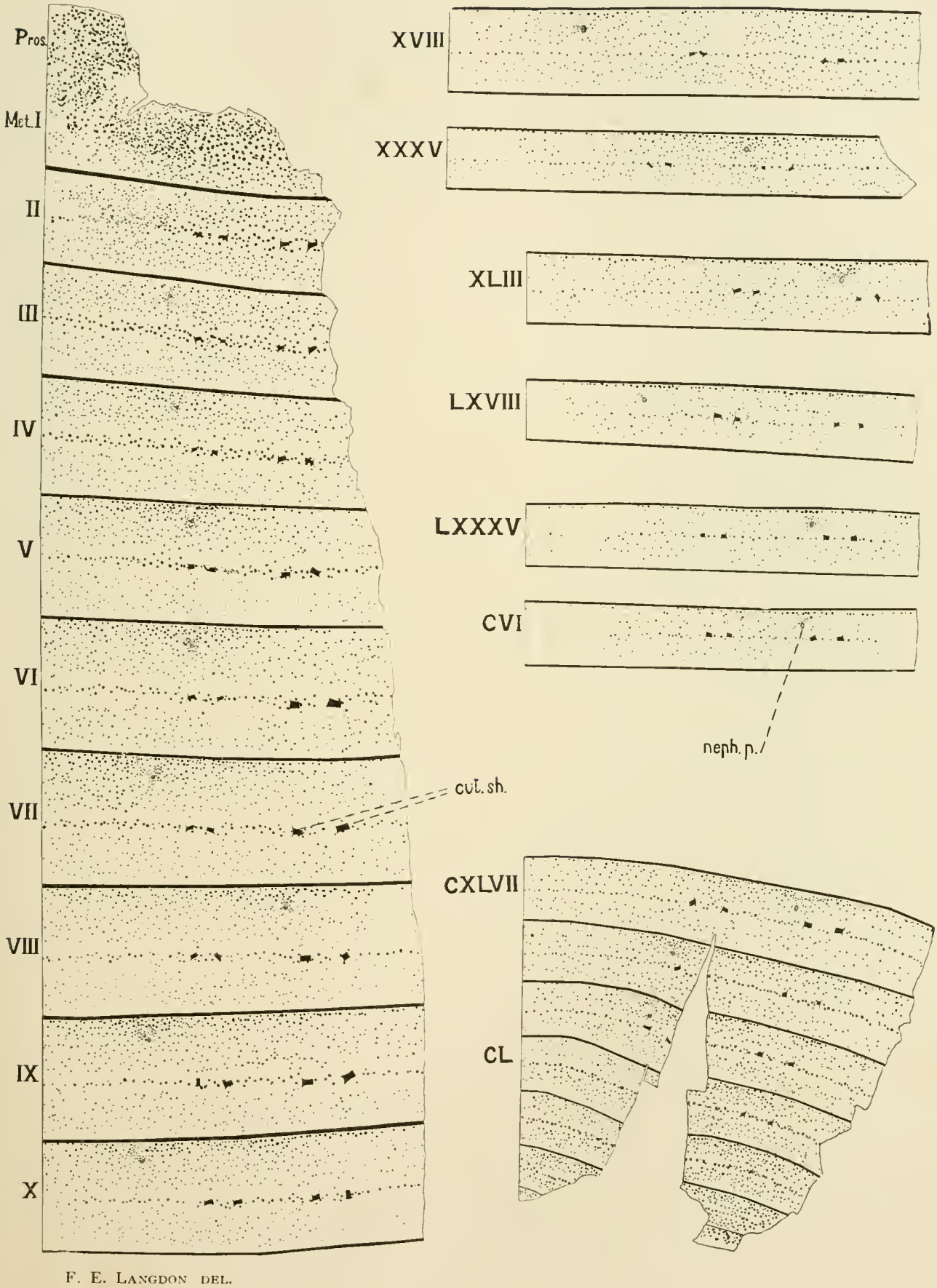




F E Langdon del.

DESCRIPTION OF PLATE XIV.

A chart showing the distribution of the sense-organs. This chart was prepared by camera drawings of characteristic metameres from the removed cuticula of one worm. The metameres are numbered along the mid-dorsal line. The jagged edge represents the mid-ventral line. The metameres are separated by heavy black lines which represent the intersegmental grooves. In each metamere the small black dots represent the cuticular spots over the sense-organs, the double circles the nephridial pores, and the pairs of black rectangular spots the cuticular sheaths of the setae. The chart was drawn to a scale of 1 dm. for 2 mm. In the plate it is very much reduced.



F. E. Langdon del.

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OF

MORPHOLOGY.

ON THE MORPHOLOGY AND PHYSIOLOGY OF THE ECHINODERM SPERMATOZOÖN.

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The present work, begun at Naples, October 4, 1892, was carried on there until March 10, 1893. It was continued at the Zoölogisches Institut of the University of Munich during the following summer, and brought to a close at the Marine Biological Laboratory, Woods Holl, Mass., during the summer of 1894.

At the Naples Zoölogical Station I occupied the "Smithsonian Table," and my heartiest acknowledgments are due to that institution, and to Professor Dohrn and his corps of assistants at Naples; also to Professor Dr. Richard Hertwig at Munich and to Professor Whitman, Director of the Marine Biological Laboratory, who have extended to me the privileges of their laboratories.

The adaptability of the cells of echinoderms to cytological studies is practically limited to the ovum; nearly all the other cells are remarkable for their small size, and although even

this smallness is in certain cases of great advantage to the investigator it has up to the present time prevented a comparative study of the spermatogenesis of the echinoderm groups. By the use of remarkably good apochromatic objectives (3 mm.: apert. 1.30 Zeiss) and compensating oculars, I have been able to study the process in several representatives of each class. The species studied included:

Stichopus regalis. (Salenka.)	Holothurioidea.
Holothuria Poli. (D. Ch.)	
Cucumaria cucumis. (*C. Planci.*)	
Antedon rosacea. (Norman.)	Crinoidea.
Echinus microtuberculatus. (Blr.)	Echinoidea.
Sphaerechinus granularis. (Ag.)	
Strongylocentrotus lividus. (Brandt.)	
Arbacia pustulosa. (Gray.)	
Echinocardium cordatum. (Gray.)	
Ophioglypha lacetosa. (Lyman.)	Ophiuroidea.
Ophiomyxa pentagona. (Müll., Fr.)	
Ophiothrix fragilis. (Müll.)	
Ophioderma longicauda.	
Chaetaster longipes. (Müll., Fr.)	Asteroidea.
Astropecten pentacanthus.	
Asterias glacialis. (O. F. M.)	
Echinaster sepositus.	
Asterias Forbesii.	

Many other species were examined. Those species which show any marked deviation from the typical dioecious condition as do, for example, the hermaphroditic forms such as *Synapta, Asterina, etc.*, or those which have become viviparous as, *e.g.*, *Amphiura squamata*, have been intentionally avoided. The other species examined were either obtainable only in limited numbers, or at the time the sexual organs were in an unfavorable condition.

As was to be expected, throughout the phylum the general aspects of the process were found to be closely similar.

In case of several species of Echinoidea which were examined the reproductive organs were found to be infested by a sort of "yellow cells," apparently an alga (see Pl. XVI), Fig. 13. So far as I am aware, these have not been described. They were found frequently in *Arbacia pustulosa*, but most abundantly in

those forms which live in the deeper water, *e.g.*, most of the spatangoids found at Naples, and particularly in *Dorocidaris papillata*. In many specimens the entire reproductive gland had become a mass of these "yellow cells." The presence of these yellow cells suggests many interesting questions. It is apparently a pathological condition and not symbiotic in the true sense. It is remarkable that here shut in within the urchin's shell they should find conditions favorable for growth.

The shape of the sexual glands in the different classes varies very considerably as a comparison of Pl. XV, Figs. 6–11, will show. The variations are coördinated with the shape of the body cavity in the different species, but the same general plan is found usually throughout the class. So that the figure given may be regarded as more or less typical for each group.

Terminology.

I have made use of the terminology, introduced by LaValette St. George now most commonly adopted. The term spermatogone = "sperm mother cell" is applied to the original cell which detaches itself from the germinal epithelium. The spermatid is the cell which changes directly into the spermatozoön. The one or more generations of cells which mediate between the spermatogone and the spermatid are the spermatocytes. As constituent parts of the cell the term cytoplasm is used for the protoplasm in distinction from the nucleus: nuclein = chromatin; caryoplasma or caryolymph = nuclear sap.

In view of the fact that the term "Nebenkern" has come to be applied to many sorts of intracellular structures including those whose morphology and physiology is known, it seems best, since the history of the middle piece or "Nebenkern" is now better understood, that this non-committal term "Nebenkern" should be replaced by some term which gives a hint as to the nature of this body. Since the middle piece or "Nebenkern" of the echinoderm spermatozoön is formed from the mitotic spindle, the term "mitosome," introduced by Platner, has been adopted and will be used to designate the middle piece, = Nebenkern = *corpuscle accessoire* of other writers.

Technique.

Owing to the extreme delicacy of the cells involved, and the extreme distortion caused by the great majority of the fixing fluids in common use for other tissues, I have endeavored to record with great care the methods which I have found best adapted. Pictet has already given figures showing the effect upon spermatozoa of the reagents most commonly employed (18). All the points described have been studied (*A*) on the fresh material, teased in sea-water on the slide. The fresh material teased on the slide was then treated with the various reagents run under the cover glass, and the reaction watched through the microscope. (*B*) The carefully killed and hardened material has been studied by dissociation methods, and (*C*) by means of sections cut in paraffin.

A. *Fresh Material on the Slide; for Structure and Development of the Spermatozoön.*

1. Neutral dahlia and methyl green.—To a watchglassful of sea-water add a small quantity of concentrated aqueous solution of dahlia. Filter very carefully, several times. Place the living spermatozoa in a drop of this liquid on the slide. After three to five minutes add a drop of a dilute solution of methyl green prepared in the same way. Examine under cover-glass. The nucleus is stained a delicate green; the mitosome and centrosome violet. This method gives a minimum distortion; (Figs. 14 and 27; compare with Figs. 5 *B* and 4 *D*); but unfortunately the results are very transitory, for after an hour or so the colors fade and finally the nucleus swells (Fig. 28) and bursts, leaving only the tail and mitosome (Figs. 53, 54) (frequently also the centrosome) visible.

If the dahlia in solution in distilled water is added to the salt water under the cover-glass, a precipitate of granules of dahlia is formed, which destroys the value of the preparation.

The value of this method lies in its delicacy, and for that reason very clean and very dilute stains are necessary. The solutions of dahlia and methyl-green in sea-water must be freshly prepared.

2. Tincture of iodine ; a very weak solution in sea-water (it must be very carefully filtered) preserves the shape and size very well. It stains the mitosome and centrosome a dark yellow or brown, the nucleus and tail a light yellow (Fig. 15).

3. Methyl-green in dilute aqueous solution drawn under the cover-glass by aid of filter-paper, stains nucleus darkly ; but after a few minutes a swelling of the heads of the spermatozoa, probably due to osmosis, begins, and the nucleus finally bursts.

4. Chloride of manganese (10% solution), add concentrated aqueous solution of dahlia ; filter. In a drop of this tease the fresh material on the slide. Examine immediately. For after a short time the heads of the spermatozoa become swollen. This reagent was particularly recommended by Pictet (18). I found it remarkably good for use in studying the mode of formation of the tail of the spermatozoön, and fairly good for the mitosome and centrosome (Fig. 24), but not so good as some other methods, notably 1 and 2.

5. *a.* Acetic acid (0.1% to 3.0%) combined with dahlia and methyl-green gave fair results. In many instances, particularly with the stronger solutions, the centrosome very gradually swelled, and slipped itself out of the cap-shaped depression in the anterior wall of the nucleus, into which it had fitted (Fig. 20). In some cases it separated completely (Fig. 22).

b. Acetic acid (33%), while preserving very well the shape of the nucleus, distorted beyond recognition the mitosome and centrosome (Fig. 23).

c. Schneider's acetic carmine. Stain for ten minutes ; decolorize in 33% acetic acid ; wash in distilled water. Examine in dilute glycerine. The size and shape of the nucleus well-preserved. There occurs a great distortion by swelling in case of the mitosome and centrosome, but this swelling is in a large measure reduced by the action of the glycerine. The nucleus is stained carmine ; the chromosomes darker. Mitosome and centrosome unstained.

6. Osmic acid is very useful for demonstrating centrosome and mitosome, particularly the former, making it very refringent.

Fix in osmic vapor, by inverting a drop of sea-water containing the teased material over the mouth of a bottle of osmic

acid (1% or 2%)). The heads of the spermatozoa become slightly distorted by swelling (Fig. 21). The osmic vapor appears to blacken the parts of the spermatozoön unequally, acting quickest upon the mitosome and centrosome.

a. Add methyl-green in solution in sea-water; after 2–3 minutes remove the surplus with filter-paper; add a drop of glycerine diluted with a weak aqueous solution of dahlia: the nucleus stains a delicate green; mitosome and centrosome violet.

b. If treated with very dilute Delafield's haematoxylin the nucleus stains deeply; the mitosome and centrosome slightly tinged.

c. Stain with very dilute aqueous solution of gentian-violet, mixed with glycerine: after a short time the mitosome and centrosome are darkly stained, the nucleus but slightly.

B. *Prolonged Fixation: Teased Material.*

This method is valuable for studying the details of spermatogenesis from the spermatogone to the mature spermatozoön. A piece of the testis is teased in a small quantity of water. Fix in Flemming's chrom-osm-acetic (strong formula), Hermann's fluid, or platinum chloride (0.3%) for 24 hours. Wash for 24 hours or more in distilled water, frequently changed and shaken. Treat with some aqueous stain. Mount in dilute glycerine or in damar. Dissociate cells by tapping cover-glass gently with the point of a needle.

7. Fix in Flemming's chrom-osm-acetic (strong formula) for 24 hours.

a. Stain with aqueous solution of safranin 10–20 minutes: only nucleus stained (Fig. 17). Add very dilute aqueous solution of dahlia: nucleus violet-red, mitosome and centrosome violet.

b. Run methyl-green under cover-glass: nucleus darkly, mitosome and centrosome slightly stained.

c. Stain in dilute aqueous solution of dahlia; add dilute methyl-green: nucleus green, centrosome and mitosome violet (Fig. 16).

d. One per cent aqueous solution of gentian-violet under cover-glass : nucleus deeply stained, mitosome and centrosome slightly.

e. Stain strongly in gentian-violet ; decolorize in water having a trace of acetic : only the nucleus is stained.

f. Gentian-violet, 24 hours ; decolorize in acid absolute alcohol ; stain 3–10 minutes with eosin in absolute alcohol : nucleus purple, mitosome and centrosome pink.

g. With very dilute Delafield's haematoxylin only the nucleus stains.

8. Fix in platinum chloride 0.3% for 24 hours.

a. Stain in aqueous solution of safranin : only nucleus stains.

b. Run dilute aqueous solution of dahlia under the cover-glass : nucleus stains red ; mitosome and centrosome violet.

C. The material for sectioning is best killed in Flemming's or Hermann's fluid. Sections in paraffin of different thickness (1–10 μ) were studied.

Historical Summary.

The history of the discovery of spermatozoa by Ham and Leeuwenhoek, and the original belief that they were animalcules — the spermatic animalcules, by some investigators regarded as infusoria, by others as allied to cercaria — need not be entered into here. It was not until about 1841 that the first important generalization upon spermatogenesis was made. Kölliker discovered that the "Spermfädern," as he called them — substituting this term for the hitherto adopted "Samenthierchen" — developed from the internal wall of the testis, and hence were metamorphosed cells (15). Later (1847) he described the spermatozoa as formed not from a single entire cell, but only from its nucleus, or even only a part of its nucleus.

As the result of a third work he decided that the spermatozoön is formed purely and simply of nuclear matter ; that the entire nucleus of the germinal epithelium cell became transformed into the spermatozoön. This nucleus, at first

spherical, elongates and divides into two parts, a denser anterior and a posterior portion. The anterior forms the head, the posterior the tail, which remains rolled up in the interior of the cell, until the spermatozoön becomes free; it then unrolls itself.

For many years following, the discussion was warmly waged between the school of Kölliker, who regarded the spermatozoön as a purely nuclear production, and those who led by Henle, Schweigger-Seidel, and LaValette St. George believed that the cytoplasm as well as the nucleus took part in the formation.

LaValette St. George by a long series of brilliant work, begun in 1867, made known the general course of spermatogenesis, and established the nomenclature now in general use. He traced the cells from the germinal epithelium, through the stages called by him the spermatogone, spermatocyte and spermatid to the mature spermatozoön.

In 1841 about the same time that Kölliker discovered that the spermatozoa are formed from the cells of the germinal epithelium, R. Wagner gave the first description of the echinoderm spermatozoön; describing that of *Holothuria tubulosa* as a "lively-motioned organism with a quite round body, and a delicate tail, similar to the Samenthierchen of the teleosts." Later observers confirmed this for other echinoderms: Quatrefages, 1842, for *Synapta inhaerens* (19); Leydig, 1852 (16), A. Baur, 1864 (1), Hamann, 1883 (10), for *Synapta digitata;* Semper, 1868 (22), for *Anapta gracilis, Chirodota incongrua,* and *Holothuria edulis;* Koren and Danielssen, 1882 (5), for *Trochostoma Thompsonii;* Jourdan, 1883 (14), and Vogt and Jung, 1887 (23), for *Holothuria tubulosa* and *Cucumaria Planci.*

Practically the first investigator to give an approximately complete account of the structure of the echinoderm spermatozoön was Jourdan (14). In addition to the head and tail (usually the only parts noticed by the earlier writers) he seems from his description to have seen not only that portion, which came to be called the "Nebenkern," "middle piece," "corpuscle accessoire," *etc.*, but even that part which will in this paper be shown to be the centrosome; he says: "at the point where

the tail begins there is a hyaline 'cupula,' distinctly marked off from the finely granular appearing protoplasm of the head." He also noticed that "after treatment with osmic acid, *etc.*, the hitherto spherical form of the head became more heart-shaped, and in the interior of this, a small shining body becomes apparent."

Others who have more or less considered points in echinoderm spermatogenesis are Selenka (21), Fol (9), Flemming (8), Jensen (13), Carnoy (2), O. Hertwig (12), Hamann (10, 11), Russo (20), Cuénot (3, 4). Recently Pictet (18) studied among that of other invertebrates the spermatogenesis in the Echinoids. But besides restricting his work to this single class, he still further limited it to the manner of the change of the spermatid into the spermatozoön. He showed that the head of the spermatozoön is formed by the entire nucleus, and is consequently composed of two substances, the nuclein (chromatin) and the caryoplasma. Further that the nuclein was no longer in separate bodies (chromosomes) but was dissolved, so to speak, in the caryoplasma to form a single homogeneous mass. That the tail of the spermatozoön is formed by the cytoplasm of the spermatid. That the "'corpuscle accessoire,' or 'Nebenkern,' is a body whose office is to eliminate from the seminal cell those substances which have become useless to the spermatozoön." As to its origin he accepts the results of Platner and Prenant.

The General Mode of Echinoderm Spermatogenesis.

The origin of the primary male sexual cells in the echinoderm group agrees with that which obtains in general in the other animal groups, namely, from the germinal epithelium lining the inner surface of the wall of the testis. I have limited my subject to the history of the germinal cells from the point where, as the spermatogones, they detach themselves from the germinal epithelium, until, as spermatozoa, the descendants of these spermatogones penetrate into the cytoplasm of the ovum in the act of fertilization. In other words, I have attempted to follow the origin and the ultimate fate of the various parts which make up the spermatozoön.

The general history is in its first and simplest aspects a series of repeated mitotic divisions. The number of these divisions in the case of echinoderms is but two. By the first the spermatogone gives rise to two spermatocytes; by the second each of these spermatocytes forms two spermatids. No later mitoses occur, and each spermatid then by a series of changes of the constituent parts of the cell become transformed into a spermatozoön. The general facts to be noted are, first, that the number of divisions is but two, instead of a large or even an indefinite number as is the case in certain animals, and as was believed to be the case in echinoderms; and secondly, that both divisions are by mitosis. The spermatogones measure 11–13μ in diameter; the spermatocytes 8–10μ; the spermatids 5–7μ, as found in teased preparations. Sections of the alveoli of the testis (Fig. 12) show zones pretty sharply characterized by the various stages in the history of the development of the spermatozoa from the spermatogones. Thus the external series of cells, *i.e.*, those next to the germinal epithelium are spermatogones with the nucleus in a resting condition, having large nucleoli. Next to this is a zone where the nuclei are in the various stages of mitosis; next a zone made up of spermatocytes; then come the spermatids, and towards the center of the lumen the spermatids in process of change into spermatozoa; then the immature, and nearer the center the ripe spermatozoa. In certain cases of starfish studied in January, nucleoli were noted in the spermatocytes: this is probably due to the fact that spermatogenesis was not going on actively at that season. Ordinarily, however, there seemed to be no resting periods intervening between the mitoses.

In the echinoderm phylum there exists a very constant and considerable difference in the shape and size of the spermatozoön of each class, and even within the class a slight difference in the shape and size of the spermatozoön of the different species. (Compare Figs. 1, 2, 3, 4 and 5.) The Holothurioidea as a group have the largest spermatozoa (Fig. 1); next in order of size come the Ophiuroidea (Fig. 4); and then the Asteroidea (Fig. 5). In these three classes the head of the spermatozoön

is approximately spherical. In the Echinoidea (Fig. 3) and in the crinoid studied (Fig. 2) (*Antedon rosacea*) the spermatozoa are smaller and the head is conical.

In many species, *e.g.*, *Holothuria Poli*, *Stichopus regalis*, *Ophioglypha lacetosa*, *A. glacialis*, *Chaetaster longipes*, and *Cucumaria planci*, in the fresh living spermatozoa there is seen a clearer, more highly refringent apex to the head (Figs. 1, 4, and 5); this has been noticed by several investigators, but so far as I am aware no one has previously worked out its origin and its significance. As will be shown below, this body is the sperm centrosome (thus confirming Cuénot's conjecture) (4).

With the exception of the cell membrane enclosing the head of the spermatozoön, and the probable presence of a minute quantity of cytoplasm, the general anatomy has already been often described, and can be readily understood from the figures; hence we may turn immediately to a consideration of the more detailed history of the various parts.

The Constituent Elements of the Spermatozoön. Their History.

In treating the subject it will perhaps be advantageous to consider first the anatomy of the mature spermatozoön, the number and nature of its constituent parts; and then to trace the history of each of these constituent parts by following its successive phases from the spermatogone, through the cell generations following (the spermatocytes and spermatids), to the mature spermatozoön; and then finally to consider the fate of each of these parts in the penetration of the spermatozoön into the egg in the fertilization process. For it is to be borne in mind that the study of spermatogenesis has shown that the spermatozoön is only a modified cell, and that each of its constituent parts has previously been a part in a preceding less specialized cell. Each, part, then should be traced from the spermatogone.

The mature spermatozoön consists of the following parts:

A. Head. { Nucleus. / Centrosome. } Head proper.
{ Mitosome. / Cell membrane. } The so-called "Nebenkern," "middle piece," "corpuscle accessoire," *etc.*
Probably also surrounding the head, and within the cell membrane a minute quantity of cytoplasm.

B. Tail.

Table of Measurements of Constituent Parts of Spermatozoa of the Species of Echinoderms Studied.

			Diameter of Nucleus	Centrosome	Mitosome
Holothurioidea	Stichopus regalis	longitudinal	4.0 μ	1.3 μ	2.0 μ
		transverse	4.0 μ		4.0 μ
	Holothuria Poli	longitudinal	3.3 μ	1.3 μ	1.6 μ
		transverse	4.0 μ		2.6 μ
	Cucumaria cucumis	longitudinal	4.0 μ	1.3 μ	1.3 μ
		transverse	4.3 μ		3.3 μ
Echinoidea	Echinocardium cordatum	longitudinal	4.2 μ	.50 μ	
		transverse	2.2 μ		2.2 μ
	Echinus microtuberculatus	longitudinal	4.0 μ	0.33 μ	1.3 μ
		transverse	2.0 μ		2.0 μ
	Spaerechinus granularis	longitudinal	3.3 μ	.66 μ	1.3 μ
		transverse	2.6 μ		2.0 μ
	Strongylocentrotus lividus	longitudinal	3.3 μ	.33 μ	
		transverse	2.0 μ		1.6 μ
	Arbacia pustulosa	longitudinal	4.0 μ	.50 μ	
		transverse	2.0 μ		2.0 μ
Crinoidea	Antedon rosacea	longitudinal	3.3 μ	.66 μ	
		transverse	1.3 μ		1.3 μ
Ophiuroidea	Ophioglypha lacetosa	longitudinal	2.6 μ	1.3 μ	2.0 μ
		transverse	4.0 μ		3.3 μ
	Ophiomyxa pentagona	longitudinal	4.0 μ	1.0 μ	1.3 μ
		transverse	4.7 μ		3.3 μ
	Ophiothrix fragilis	longitudinal	2.6 μ	1.0 μ	1.3 μ
		transverse	2.8 μ		3.3 μ
	Ophioderma longicauda	longitudinal	3.3 μ	1.0 μ	2.0 μ
		transverse	4.3 μ		3.3 μ
Asteroidea	Chaetaster longipes	longitudinal	2.6 μ	1.2 μ	2.0 μ
	Astropecten pentacanthus	longitudinal	2.8 μ	1.0 μ	1.1 μ
		transverse	3.3 μ		2.2 μ
	Asterias glacialis	longitudinal	2.6 μ	1.3 μ	1.3 μ
		transverse	3.3 μ		2.0 μ
	Echinaster sepositus	longitudinal	2.0 μ	1.2 μ	1.3 μ
		transverse	3.3 μ		1.7 μ
	Asterias Forbsii	longitudinal	3.3 μ	1.2 μ	1.3 μ
		transverse	4.0 μ		3. μ

The Nucleus.— The nuclei of the spermatogones in the outer zone, as seen in a section of the testis, usually alone of all the cells show nucleoli. The nucleus is large relatively to the amount of cytoplasm. It very soon begins the process by which it will ultimately give rise to the spermatozoa. It divides by mitosis and forms the nuclei of two spermatocytes. The number of chromosomes into which the nuclein of the spermatogone collects seems to be 28–36 : in the spermatocyte 16–18. The attempt to count with exactness so small and so numerous bodies, so closely crowded together, is well nigh fruitless. The dividing nucleus plainly is seen to be made up of several substances, *e.g.*, nuclein, staining deeply with methyl green; the caryoplasma staining lightly; and another substance which has the appearance of minute granules; these evidently form the mitotic spindle. A centrosome is also present. These granules and the centrosome take a violet color with dahlia (Fig. 29).

The nucleus of each spermatocyte has the same constituent parts as that of the spermatogone. It divides by mitosis and forms the nuclei of two spermatids. The nucleus of each spermatid contains eight or nine chromosomes, and caryoplasma. Within the nucleus there seems to be no signs of the granules which formed the nuclear spindle; but these granules and the centrosome are now very distinctly seen to be in the cytoplasm (Figs. 32, 33). It should be noted that each of these mitoses are "reducing divisions."

With the spermatid begin those changes in the shape and constitution of the nucleus which are connected with the specialized form of the spermatozoön. (1) The change in shape. In the case of the Holothurioidea, Asteroidea, and Ophiuroidea the change is insignificant, usually only a slight flattening in the antero-posterior direction. But with the Crinoids and with the Echinoidea the nucleus gradually changes from spherical to conical. (2) There seems to be a change in the constitution of the nucleus, as remarked by Pictet (18). I can confirm his observation. The chromosomes (usually nine, sometimes eight) can be demonstrated in the spermatids and in certain of the spermatozoa, probably the immature ones. But in others the

nucleus remains homogeneous under the same reagents and conditions which demonstrate the chromosomes in the others. These spermatozoa with the homogeneous nuclei are the most active and most frequently penetrate into the ovum. In sections of fertilized eggs the nucleus of the spermatozoön when in the outer zone of the cytoplasm of the ovum is small, dense, and homogeneous (Fig. 56); on the other hand the one which has traveled some distance toward the female pronucleus is considerably larger, and shows the eight or nine chromosomes surrounded by a lightly staining caryoplasma (Figs. 57, 58). Hence it is probable either that the chromosomes in the nucleus of the spermatozoön dissolve in the caryoplasma and form a denser homogeneous mass preparatory to penetrating the membranes and more compacted outer cytoplasmic layer of the ovum, or else the caryoplasma is extruded and the nucleus of the mature spermatozoön consists very largely or even solely of chromosomes (nuclein) closely packed together. There is a very considerable reduction in the size of the nucleus in changing from that of the spermatid to that of the spermatozoön, and correspondingly an increase in size, with a reappearance and wider separation of the same number of chromosomes, after the spermatozoön in the fertilization process has passed the peripheral denser portion of the cytoplasm of the ovum. The question is whether this change in the nucleus is merely one of density, *i.e.*, (1) does the nucleus of the mature spermatozoön consist of the same quantity of nuclein and caryoplasma, but with the nuclein dissolved in the caryoplasma, or (2) does the caryoplasma as a liquid portion pass into the cytoplasm before the spermatozoön becomes fully mature, and after the spermatozoön has entered the cytoplasm of the egg is this caryoplasma restored from the cytoplasm of the egg? On account of the very evident alterations in size of the nucleus, one is inclined towards the latter alternative. It seems to be a pretty good case for proving that an interchange of substance goes on between the nucleus and the cytoplasm, and that in this instance at least the substance which passes through the nuclear membrane is the caryoplasma, a liquid protoplasm. If this is the case, it makes strongly for the view that the nuclein is the

essential part, for in this case most of the male caryoplasma passes out into the cytoplasm of the male cell, and the caryoplasma is replaced by liquid from the cytoplasm of a female cell.

The nucleus varies considerably in size and shape in the different classes, as a comparison of the table, p. 246 and figures (1–5) will show. In *Antedon* and the Echinoidea it is comparatively small; while larger in the Asteroidea it is largest in the Holothurioidea and Ophiuroidea. In the anterior surface, usually at the very apex, is a depression into which the centrosome fits. In the Echinoidea, however, it is often not at the very tip of the sharp-pointed conical nucleus, but on the side, usually, however, very near the anterior end (Figs. 24, 25, 26).

Centrosome. — I have never seen a centrosome in the spermatogones in the outer zone, *i.e.*, next to the germinal epithelium. I first succeeded in finding it in the dividing spermatogones. At first it seemed to be within the nuclear membrane. But observation on this point is very difficult, and I am by no means positive in regard to the manner or place of first appearance. I have never seen in the spermatogone the actual division of the centrosomes, but it is probable that it occurs preparatory to the mitotic division. With the disappearance of the nuclear membrane the centrosomes are seen to have the usual position at the poles of the nuclear spindle. This spindle seems to be formed of those violet-staining granules which are present in the nucleus before the disappearance of the nuclear membrane (Figs. 29, 30, and 31). As shown in Figs. 30 and 31, these granules remain in close proximity to the chromosomes, the cytoplasm being entirely free from them, previously to the final division, which gives rise to those cells, the spermatids, which will become the spermatozoa. After the division of the nucleus of the spermatocyte into the two spermatid nuclei, and before the division of the cytoplasm has taken place, those granules which composed the spindle fibres are seen scattered through the cytoplasm (Figs. 32, 33); the nuclei themselves, rounded up and with a nuclear membrane, are free from the granules. In the cytoplasm also, close beside each nucleus remains one of the centrosomes, which took part in the division of the cell (Figs. 32, 33, 34, 35).

The probable history of the centrosomes is as follows : the original centrosome appears first in the spermatogone and is of intranuclear origin (I cannot say that the centrosome is not present in the spermatogones while they are still close to the germinal epithelium, but I have never seen it previous to the disappearance of the nucleolus ; and when first seen it seems to be within the nuclear membrane). In the spermatogone it divides into two, which participate in the mitotic division resulting in two spermatocytes. With each spermatocyte nucleus there is left one centrosome, which is one-half of the original centrosome of the spermatogone. Initiatory to the mitotic division of the spermatocyte, this centrosome divides into two, which come to lie at the poles of the spindle. With the completion of this mitosis each spermatid contains one centrosome. Thus from the original centrosome of the spermatogone four centrosomes have been derived, and each of these four centrosomes comes to be placed at the apex of the head of a spermatozoön, and in the fecundation process enters the ovum with the nucleus of the spermatozoön and takes part in the subsequent fertilization.

When the nuclear membrane is formed around the nucleus of the spermatid the centrosome is not included, but is left outside in the cytoplasm (Figs. 34, 36, 41, 43, 45). This fact either points to the probability that the centrosome is extranuclear in the spermatogone, or else that for some reason the condition in the spermatogone varies from that in the spermatid. If the latter is true, a possible explanation for this difference may be found in the fact that with the spermatid the series of mitotic divisions is completed, and that subsequently the constituent parts of the cell will undergo an extreme modification.

In the light of the work of Fol, Guignard, and Conklin on the part which the centrosome takes in the process of fertilization, it is not difficult to see why the centrosome should be extranuclear in the ovum and in the spermatozoön.

In view of the fact that the centrosome is certainly extranuclear in the spermatid and in the spermatozoön, it is only proper to question whether it is not so also in the

spermatogone. Study of some more favorable forms will decide this.

With the formation of the tail it first begins to be possible to say which part of the spermatid will be the anterior point of the spermatozoön. The centrosome, which hitherto apparently has had no special position with reference to the nucleus (Figs. 34, 39), now comes to lie close beside it, and, except in the Echinoidea, usually directly opposite to the point where the tail is forming (Figs. 35, 36, 40, 42, 43).

With the diminution of the cytoplasm around the nucleus, the centrosome comes to lie in a depression in the nucleus, but remains entirely outside of the nuclear membrane. This condition is probably brought about mechanically by the pressure of the tightly drawn cell-membrane which invests the spermatozoön, pushing the unyielding refringent centrosome into the wall of the nucleus.

In the Asteroidea examined the centrosome consists of two distinct parts or substances; a clear, transparent, lightly staining part surrounding a dumbbell-shaped body deeply staining with dahlia (Figs. 14, 22, 41, 49, 51). This dumbbell-shaped body reminds one of the figures given by various investigators for the first stage in the division of the centrosome.

As the table (p. 246) shows, the size of the centrosome varies widely in the different classes, and even in the different species within the class. It is largest in the Holothurioidea, and smallest in the Echinoidea. Of the Echinoidea examined it is smallest in *Echinus microtuberculatus* (Fig. 46), where its diameter is only about equal to the diameter of the tail of the spermatozoön. The very small size of the centrosome in the Echinoidea is probably the reason why it seems to have been overlooked by Pictet and others, who have worked on these forms alone.

By means of those reagents which cause a slight swelling of the nucleus or of the centrosome, notably chloride of manganese, dilute acetic acid, *etc.*, the centrosome can be made to slip out of the little depression in the surface of the nucleus (Fig. 20), though it is still held close to the nucleus by the cell membrane which surrounds the entire spermatozoön. The centrosome preserves its shape very well with most reagents,

strong acetic acid excepted (Fig. 23); even when the nucleus swells and bursts, the centrosome as well as the mitosome remains uninjured.

In the living spermatozoön in sea-water the centrosome can be seen as a brighter, more refringent spot near the anterior end, in the case of the Holothurioidea, Asteroidea, and Ophiuroidea examined (Figs. 1, 4, 5). This fact was noticed by Cuénot (4). It was in 1883 seen by Jensen (13), but he interpreted it as merely a depression at the anterior end of the nucleus caused by the shrinkage due to expulsion of the achromatin from the nucleus.

As to the further history of the centrosome : the sperm centrosome was seen and figured by O. and R. Hertwig, Bovieri, and Fol, in the fertilized echinoderm egg. But, so far as I am aware, no one has previously worked out the history of this centrosome. The Hertwig brothers believed that it came from the middle piece, since it showed the same micro-chemical reactions. Fol believed that the anterior portion of the nucleus became fragmented off. Pictet pointed out the erroneousness of Hertwigs' and inclined to Fol's view. But, as shown above, the sperm centrosome is the centrosome of the spermatid, and of the previous cell-generations, and it continues in company with the nucleus of the spermatozoön until the completion of the process of fertilization. As soon as it has passed the denser outer layer of the cytoplasm of the egg it draws farther away from the nucleus, and the characteristic radiations appear in the cytoplasm. It no longer directly precedes the nucleus, but comes to lie at one side (Fig. 58).

The Mitosome. — The very small granules, darkly staining with dahlia, visible in the nucleus before the disappearance of the nuclear membrane (Fig. 29), and which later become the fibres of the mitotic spindle (Fig. 31), have been already referred to (p. 246); as well also the fact that after the division of the spermatocyte into the spermatids, these granules, which were the mitotic spindle of the spermatogone and spermatocyte, are no longer included within the nuclear membrane, but are scattered through the cytoplasm (Figs. 32, 33, 34). With the beginning of those changes which mark the transformation of

the spermatid into the spermatozoön, these granules gradually fuse into larger and larger bodies (Figs. 34, 35, 36, 39*m*), until they come to form in the cytoplasm a small number (2–8) of refringent spheres (Figs. 35, 36, 49*m*): these finally fuse into a single spherical mass, the mitosome (Figs. 41, 42, 43*m*). The mitosome apparently may at first take any position whatever in the cytoplasm, but with the formation of the tail and the consequent gradual diminution of the cytoplasm in the head of the spermatozoön the mitosome soon becomes pressed upon by the cell membrane, and is gradually drawn into the place of least resistance, *i.e.*, between the nucleus and the beginning of the tail, its normal position in the mature spermatozoön.

LaValette St. George's discovery in 1867 of this body, named by him the "Nebenkern," introduced new questions into cellular morphology and physiology. What is and whence comes this body? What purpose does it subserve? The discoverer at first held that this "Nebenkern" is formed by the condensation of a part of the protoplasm of the spermatid, *i.e.*, that it is of cytoplasmic origin (36). This view was adopted by Metschnikoff, Balbiani, and Bütschli from results obtained from their investigations upon various Arthropods, and by Keferstein upon Molluscs.

According to the more recent researches of LaValette St. George, Platner, Prenant, and myself, the "Nebenkern" is formed from the granules which are the remains of the nuclear spindle, and which after the final mitotic division fuse into a single large spherical mass.

As to what part of the mature spermatozoön is formed by the "Nebenkern," there seems to have been a great disagreement among investigators, not only in the different animal groups, but even in many cases with different species within the same group. According to the view of Keferstein (33), LaValette St. George (36), Metschnikoff (43), and Duval (27, 28), for Molluscs, particularly the Pulmonates; and of Grobben (30) for the Decapods, the "Nebenkern" forms the head of the spermatozoön. According to the observations of LaValette St. George (38), and Bütschli (25), upon Insects, and of Pictet (18) and myself upon Echinoderms it forms the middle piece.

Prenant (45) finds that in the Reptiles it forms a cap to the head ("la coiffe céphalique"), but that in the Pulmonates it is dissolved in the cytoplasm which finally forms the tail of the spermatozoön.

From the fact that the "Nebenkern" does not in all species act in exactly the same manner in the building up of the spermatozoön, but apparently sometimes the major part becomes placed anteriorly, sometimes posteriorly to the nucleus proper, the earlier investigators have overlooked the smaller portion and hence thought that the head alone or the middle piece alone was formed from the "Nebenkern," *i.e.*, that the mitosome passed entirely into the head or into the middle piece as the case might be. But Platner's observations upon Pulmonates and these upon Echinoderms may put the question in a clearer light. In many cases, *e.g.*, in the Pulmonates according to Platner, the "Nebenkern" comes to form a considerable part of the spermatozoön anterior to the nucleus proper; the rest of the "Nebenkern" forms the middle piece. He found that in the spermatid the "Nebenkern" is made up of two portions, a part, the "mitosoma," surrounding a much smaller body, the "centrosoma." In the change of the spermatid into the spermatozoön these two parts separate; the centrosome part takes a position anterior, but the mitosome a position posterior to the nucleus (*i.e.*, becomes the middle piece). Now in the Echinoderm spermatid the centrosome is at no time contained within the mitosome, but the ultimate arrangement of these parts in the mature spermatozoön is the same as in the Pulmonates. The fact, then, that not only the middle piece but also a part of the head proper is formed from the "Nebenkern" largely explains the cause of so many apparently disagreeing results.

This comparison of the conditions obtaining in different groups in regard to the "Nebenkern," and the relations of mitosome and centrosome, together with the fact that mitosome and centrosome throughout their history have identical micro-chemical reactions (compare Figs. 14 to 28), has led me to infer that they are differentiations of the same substance, yet so well specialized that they subserve specifically distinct func-

tions: that the sperm centrosome is that portion of the mitosome material which must be transmitted with the nuclear material in the act of impregnation in order to initiate the ontogeny of a new individual. The differentiation into mitosome and centrosome must have taken place previous to the division of the spermatogone. The question as to whether the nuclei are or are not the sole participants in fertilization depends to a considerable extent upon the decision of the question of the nuclear or the cytoplasmic origin of the mitosome and centrosome. It is clearly not sufficient to show that the mitosome or centrosome is in the nucleus or in the cytoplasm in any certain cell, but the point must be confirmed by reference to as large a number as possible of successive cell generations.

As to what is the fate of the mitosome in the process of fertilization there has been a wide range of opinions, and if we may judge from the great number of divergent observations, its fate is quite different in the various animal groups, and much careful work is still necessary on this point. Observations upon its fate in the case of the Echinoderms have been made by Selenka (21), Pictet (18), Cuénot, and myself. According to Selenka's account, the mitosome becomes swollen, and moving towards the female pronucleus, finally fuses with it ; while the nucleus and the tail are absorbed.

The view which Pictet brought forward, and which seems to be confirmed by my preparations, is that the mitosome breaks away from the nucleus, and that while the nucleus and the centrosome proceed on towards the female pronucleus, the mitosome and tail are left close to the point where the spermatozoön entered the cytoplasm of the egg, and there break down and are absorbed. The observations of Pictet and Cuénot appear to rest largely upon the fact that the mitosome was seen in some cases previously to the penetration of the spermatozoön into the egg, to break off from the spermatozoön, and that such spermatozoa, lacking the mitosome, are quite capable of normal fertilization. My results are based upon sections of artificially fertilized eggs of *Asterias glacialis*.

At this point it may be well to add that though I have a very great number of times observed carefully the mode of

penetration of the Echinoderm spermatozoön into the egg, I have never seen the cytoplasm of the egg send up a little projection to meet the incoming spermatozoön, as described by Fol, and now incorporated into all the text-books. On the contrary one sees a depression in the surface of the cytoplasm caused by the mechanical pressure of the spermatozoön; exactly the same condition as when one presses a sharp pin into a piece of rubber. The path made by the nucleus as it plows through the cytoplasm is plainly visible (Fig. 57).

The female pronucleus moves towards the advancing male pronucleus in this way: at first spherical, it takes on a pear shape, the narrower apex being directed towards the male pronucleus. This anterior projecting process reminds one of a very large, blunt pseudopodium of an amoeba; and the pronucleus travels by an almost amoeboid motion; the shape of the nucleus constantly changing, the projection being sent out and the posterior portion pushing forward and filling it out.

Tail.—The tail, a round flagellum about 0.2 μ, 0.3 μ in diameter varies in length not only in the different groups, but also in different individuals of the same species. In the species studied the extremes are found among the Holothurioidea; in *Stichopus regalis* it is very short, about 40.0 μ, while in *Holothuria Polo* it is about 90.0 μ. The other species studied showed all intermediate lengths.

As Pictet has already shown for the Echinoidea (18) the tail is formed from the cytoplasm of the spermatid. Although I can add but little to the results obtained by him, more than to confirm them for representatives of all the classes of Echinoderms, yet for the sake of more fullness I will go over the subject here. Soon after the mitotic division of a spermatocyte into two spermatids, the cytoplasm of the spermatid begins to form a bulging which increases into a large projection like an enormous blunt pseudopodium (Fig. 40); the cytoplasm continues to push or flow into this projection and it becomes elongated and flask-shaped, the body of the flask consisting of a large drop of cytoplasm, which is connected with the cytoplasm proper by a narrow neck (Figs. 42, 43). The continued lengthening of the tail takes place with the elongation and

diminution in the diameter of this neck, together with a diminution in the size of the drop of cytoplasm at the tip, as well also by a diminution of the cytoplasm which has hitherto remained around the nucleus and the mitosome in the cell proper (Figs. 35, 36, also Figs. 40, 42, 43, 44, 45, 50). The tail then is the cytoplasm of the spermatid, which has become modified in a very special way. It is possible that its violent motions in the water are expressions of some molecular change which the sea-water brings about in the protoplasm of the tail, for I have often noticed that spermatozoa when first removed from the testis into sea-water lie motionless; after a short time a slight motion begins, which after a few minutes increases to the normal rapid motion.

Pictet concluded that the tail is attached to the posterior part of the nucleus, but certain facts seem to point otherwise, for in those cases where the nuclei are caused to swell and finally burst, the tail is almost invariably left attached to the mitosome (Figs. 53, 54). Further in the process of fertilization, the mitosome and the tail together separate from the nucleus (Figs. 56, 57). On the other hand, however, is the observation made by Pictet and Cuénot, referred to above, that in many cases, apparently with one species, *Asthenosoma*, as the rule, the mitosome becomes detached from the nucleus before the spermatozoön penetrates the egg. It seems most probable that the tail is in direct continuation with the cell membrane which surrounds the spermatozoön. The cell membrane being morphologically but the external slightly changed cytoplasm, probably differs very little, morphologically not at all, from the tail, and these two, the tail and the cell membrane, probably pass insensibly into one another; the mode of development would seem to prove this.

Cell membrane. — A delicate cell membrane surrounds the head of the spermatozoön, inclosing the nucleus, centrosome, and mitosome. This membrane is best seen in cases where from some mechanical cause a slight separation has taken place between the nucleus and the mitosome; this membrane in that case being stretched but still unbroken (Fig. 55). It can also be seen stretching over the centrosome in cases where the

centrosome has been crowded out of the socket in the anterior end of the nucleus (Fig. 20). In certain preparations which I made of spermatozoa, killed in osmic vapor, stained in Delafeld's haematoxylin, and mounted in dilute glycerine, after a time the nuclei burst; a portion of the darkly stained nuclear contents escaped, leaving the cell-membrane distinctly visible. I regard this membrane as the original cell membrane which has persisted from the spermatid. Pictet (18) thinks that this membrane dissolves and with the cytoplasm contributes to the formation of the tail, but in the case of the Siphonophores he found that it persisted, but he adds that "it is probable that it ultimately dissolves at the moment of fecundation."

Conclusion.

Many of the facts found in this study of Echinoderm spermatogenesis range themselves with a large mass of facts which are being accumulated from all branches not alone of the animal, but also of the vegetable realm, tending still more to strengthen the theory first advanced by E. L. Mark, and lately confirmed by O. Hertwig (32) and others that the polar bodies are aborted eggs; that there is a close parallelism between the histories of the cells concerned in the formation of the egg and of the spermatozoön; that the egg, the spermatozoön, and the three polar bodies are strictly homologous; and that any difference apparent is to be regarded as a specialization for specific purposes; the accumulation of all of the food yolk in one of the ova results in the uselessness of the other three (the polar bodies); the modification of the four spermatids, derived from the spermatogone (which is the homologue of the unmaturated ovum) by differentiation of the cytoplasm into a vibratile tail, the separation and subsequent extrusion of the no longer useful material of the nuclear spindle, in the form of the mitosome, are modifications merely of parts of the cell. (It is hardly necessary to call attention to the absolute absence of homology between the extrusion of the "polar bodies" and the extrusion of the "mitosome.")

Cases where the conditions lie so simply are by no means common. But investigation will show that it is much more general than has been supposed, and that cases where the spermatogone gives rise to four spermatozoa have been found in widely different groups, in many Vertebrates, Pulmonates, Lepidoptera and other Arthropods, and Ascaris (similar conditions also are found in the pollen formation in many plants); hence we are led to consider the probability that this condition, which is well exemplified in the case of the Echinoderm, is the simple ancestral condition, from which the great variety of types of spermatogenesis have been derived in adaptation to the various modes of life of the different animals and plants; the necessity for a greater or a lesser number of spermatozoa being met by an alteration in the number of cell divisions between the spermatogone and the spermatozoön. The great majority of the Echinoderms have continued in this primitive condition: the number of spermatozoa being at least four times the number of the eggs, since each spermatogone, the homologue of the unmaturated egg, gives rise to four spermatozoa, but further from the fact that the spermatogones being very much smaller than the ova, an enormously greater number can be contained in the testes. This numerical ratio between the ova and the spermatozoa has been sufficient to maintain the race, particularly since the conditions governing the existence of the Echinoderms have undergone remarkably slight changes from the time of their first appearance up to the present day.

The demonstration of the close similarity between the histories of the male and female cells throughout the animal and vegetable kingdoms will render easier the understanding of hermaphroditism and its kindred subjects.

In closing, I will call attention to the following points:

1. The size and shape of the spermatozoa differ in the various classes. In the Holothurioidea, Ophiuroidea, and Asteroidea, the head is spherical; in the Crinoidea and Echinoidea it is conical.

2. A cell membrane completely surrounds the spermatozoön. The tail is in connection with this cell membrane, and not attached directly to the nucleus or to the middle piece.

3. The number of spermatozoa formed from a spermatogone is four, by means of two mitotic divisions.

4. Each mitotic division from the spermatogone to the spermatid is a "reducing division," *i.e.*, the number of chromosomes is reduced one-half by each division.

5. The number of chromosomes in the spermatozoön is nine. This number is characteristic for the Echinoderm phylum.

6. The nucleus of the spermatid contains chromosomes and caryolymph. When this nucleus becomes the nucleus of the mature spermatozoön chromosomes and caryolymph are not distinguishable. They have either mingled to form a homogeneous mass, or else the caryolymph has been extruded. The smaller size of the nucleus of the spermatozoön points to the latter alternative.

7. When the nucleus of the spermatozoön in the fertilization process has passed the outer denser cytoplasmic portion of the ovum it increases in size; caryolymph and chromosomes appear again. Hence, one would infer that the male pronucleus derived its caryolymph from the cytoplasm of the ovum.

8. The small refringent body seen by various investigators at the apex of the head of the spermatozoön is shown to be the centrosome. It is derived directly from the centrosome of the previous generations of cells. It is extranuclear in position in the spermatid and spermatozoön; possibly intranuclear in the spermatogone and spermatocyte.

9. The sperm-centrosome, with its definite spherical outline, consists of two sorts of substances, a denser central portion surrounded by a clearer homogeneous mass. I have never succeeded in finding the egg centrosome.

10. The sperm-centrosome is very small in Crinoidea and Echinoidea; much larger in Holothurioidea, Ophiuroidea, and Asteroidea. Since throughout it has the same optical appearance and shows the same micro-chemical reactions as the mitosome (Nebenkern; Mittelstück; middle piece), and also from a comparison with the conditions found elsewhere, particularly in the Pulmonates (where in the spermatid the centrosome is contained within the mitosome, but in the mature spermato-

zoön, the centrosome becomes placed anterior, the mitosome posterior to the nucleus [Platner]), it seems probable that centrosome and mitosome are differentiations of one and the same substance. This is the same conclusion reached by Watasé (46) arguing on another line.

11. The mitosome and centrosome represent the material of the nuclear spindle (cytomicrosomes). The mitosome is that portion which is of no further use, while the centrosome is that part which enters with the nucleus, and is either the necessary complement of the centrosome and spindle of the ovum in fertilization, or else it alone constitutes the mechanism for initiating cleavage. The differentiation of mitosome and centrosome may be a device for overcoming the mechanical difficulties of transferring a large quantity of spindle-forming substance through the egg membranes and denser outer-cytoplasmic portion.

12. The mitosome takes no part in the fertilization process, but together with the tail remains near the periphery of the egg, and is rapidly broken down and absorbed; while the nucleus and centrosome of the spermatozoön push on towards the nucleus of the egg.

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Woods Holl, Mass.,
Aug. 24, 1894.

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DESCRIPTION OF PLATES.

Abbreviations.

al.	alveolus.	*n.*	nucleus.
c.	centrosome.	*no.*	nucleolus.
ch.	chromosome.	*p.*	peritoneum.
c.t.	connective tissue.	*sc.*	spermatocyte.
cy.	cytoplasm.	*sd.*	spermatid.
m.	mitosome (Nebenkern = middle piece = corpuscle accessoire).	*sg.*	spermatogone.
		sn.	spermatozoön.

All figures except 6, 7, 8, 9, 10, 11, 12, and 13 are magnified 1500 diameters. Camera drawings, with Zeiss 3 mm., 1.30 apert. apochrom. hom. immers. objective, and compensating ocular 18. All the details so far as possible are drawn to scale. The coloring was done directly from the preparations.

Only in Figs. 1, 2, 3 A, 4 A, 5 C and D, 35, 36, 40, 42, 43, 44, 45, 48, 50, and 55 is the length of the tail represented.

EXPLANATION OF PLATE XV.

FIG. 1. Ripe living spermatozoa of Holothurioidea. A. *Holothuria Poli.* B. *Cucumaria cucumis.* C. *Stichopus regalis.*

FIG. 2. Ripe living spermatozoa of Crinoidea. *Antedon rosacea.*

FIG. 3. Ripe living spermatozoa of Echinoidea. A. *Strongylocentrotus lividus.* B. *Sphaerechinus granularis.* C. *Echinus microtuberculatus.* D. *Echinocardium cordatum* (a spatangid). E. *Arbacia pustulosa.*

FIG. 4. Ripe living spermatozoa of Ophiuroidea. A. *Ophiomyxa pentagona.* B. *Ophioglypha lacetosa.* C. *Ophioderma longicauda.* D. *Ophiothrix fragilis.*

FIG. 5. Ripe living spermatozoa of Asteroidea. A. *Echinaster sepositus.* B. *Asterias glacialis.* C. *Chaetaster longipes.* D. *Astropecten pentacanthus.*

FIG. 6. Diagram of testis of *Ophioglypha lacetosa* (Ophiurid).

FIG. 7. Diagram of testis of *Astropecten pentacanthus* (Asterid).

FIG. 8. Diagram of testis of *Cucumaria cucumis* (Holothurid).

FIG. 9. Diagram of testis of *Asterias glacialis* (Asterid).

FIG. 10. Diagram of testis of *Stichopus regalis* (Holothurid).

FIG. 11. Diagram of testis of *Brissus unicolor* (Echinid).

FIG. 12. A portion of a section of an alveolus of the testis, showing the general mode of spermatogenesis ; the spermatogone (*sg.*) being next to the germinal epithelium, the mature spermatozoa (*sn.*) in the center of the lumen of the alveolus. Many of the cells are omitted for the sake of clearness.


1.
c
n
m
A
B
C
2
n
m
3.
n
m
A
B
C
D
E
4.
c
n
m
A
B
C
D
5
c
n
m
A
B
C
D
6.
al
7
8
12.
p
ct
no
sg
sg
sc
sc
sd
sd
sc
sd
sn
sn
9.
al
10.
11.

EXPLANATION OF PLATE XVI.

FIG. 13. "Yellow cells" from testis of *Dorocidaris papillata*. *A*, fresh, living. *B*, stained with methyl green. *C*, stained with dahlia.

FIG. 14. Spermatozoön. *Asterias glacialis*, sea-water + dahlia; sea-water + methyl green.

FIG. 15. Spermatozoön. *Asterias glacialis*, dilute tincture of iodine in sea-water.

FIG. 16. Spermatozoön of *Asterias glacialis*. Flemming (strong formula) 24 hours: water 24 hours, dahlia, methyl green, glycerine.

FIG. 17. Spermatozoön of *Asterias glacialis*. Flemming (strong formula) 24 hours: water 24 hours, safranin 15 minutes.

FIG. 18. Spermatozoön of *Asterias glacialis*. Flemming (strong formula) 24 hours: water 24 hours, Delafield's haematoxylin.

FIG. 19. Spermatozoön of *Asterias glacialis*. Flemming (strong formula) gentian violet (24 hours); acid absolute alcohol: eosin in abs. alcohol 3 minutes, clove oil.

FIG. 20. Spermatozoön of *Astropecten pentacanthus*. Very dilute acetic + dahlia; after 5 minutes. The centrosome and mitosome swell slightly.

FIG. 21. Spermatozoön of *Astropecten pentacanthus*. Osmic vapor; dahlia; glycerine.

FIG. 22. Spermatozoön of *Chactaster longipes*. Acetic 3%; methyl green + dahlia. The centrosome has separated from the depression in the wall of the nucleus, and has become entirely free from the spermatozoön.

FIG. 23. Spermatozoön of *Chaetaster longipes*. Acetic 33%; methyl green. A great distortion of the centrosome and mitosome results.

FIG. 24. Spermatozoön of *Strongylocentrotus lividus*. Chloride of manganese 10% + dahlia.

FIG. 25. Spermatozoön of *Echinocardium cordatum*. Dilute gentian violet in sea-water.

FIG. 26. Spermatozoön of *Echinocardium cordatum*. Chloride of manganese 10% + dahlia. The normal shape of the nucleus is somewhat altered by the reagent.

FIG. 27. Spermatozoön of *Ophiothrix fragilis*. After 25 minutes in sea-water + dahlia.

FIG. 28. Spermatozoön of *Ophiothrix fragilis*. After one hour in sea-water + dahlia. The nucleus has swollen considerably.

FIG. 29. Spermatogone: *Stichopus regalis*. (NOTE. — Figs. 29 to 34 upon treatment with Flemming (24 hours); water (24 hours); methyl green and dahlia; glycerine.) The nucleolus has disappeared. The nuclear membrane still persists. Two centrosomes are present.

FIG. 30. Spermatocyte: *Stichopus regalis*.

FIG. 31. Spermatocyte: *Stichopus regalis*. In mitosis; all of the chromosomes are not shown in the drawing. (NOTE. — The line from *cy* should go to the spindle. No nuclear membrane present.)

FIG. 32. Spermatocyte: *Stichopus regalis*. After division of the nuclei to form two spermatids. The cytomicrosomes (*cy*) which formed the spindle, previously inside the nucleus, are now in the cytoplasm. (NOTE. — In the upper cell the letters, *c* and *n*, should be reversed.)

FIG. 33. Spermatocyte: *Stichopus regalis.* After division of the nuclei to form two spermatids. The cytomicrosomes which formed the spindle, previously inside the nucleus, are now in the cytoplasm.

FIG. 34. A Spermatid of *Stichopus regalis.* The cytomicrosomes are fusing into larger masses, *m*, which will ultimately fuse and form the mitosome. The centrosome is plainly in the cytoplasm.

FIG. 35. A metamorphosing Spermatid of *Stichopus regalis.* Sea-water + dahlia and methyl green preparation. Shows a later stage in the formation of the mitosome, and of the tail.

FIG. 36. A metamorphosing Spermatid of *Stichopus regalis.* Sea-water + dahlia and methyl green preparation. Shows a later stage in the formation of the mitosome, and of the tail.

FIG. 37. Spermatogone: *Asterias Forbsii.* Flemming (24 hours); distilled water (48 hours); Delafield's haematoxylin; dilute glycerine.

FIG. 38. Spermatocyte: *Asterias Forbsii.* Flemming (24 hours); distilled water (48 hours); Delafield's haematoxylin; dilute glycerine.

FIG. 39. Spermatid: *Ophyoglypha lacetosa.* Sea-water + dahlia and methyl green.

FIG. 40. Metamorphosing Spermatid of *Ophiothrix fragilis.* Platinum chloride 0.3% (12 hours): dahlia and methyl green. Shows mode of formation of mitosome, and of the tail.

FIG. 41. Spermatid of *Chaetaster longipes.* Platinum chloride 0.3% (12 hours); dahlia and methyl green. Shows mode of formation of mitosome, and of the tail.

FIG. 42. Metamorphosing Spermatid: *Chaetaster longipes.* Platinum chloride 0.3% (12 hours); dahlia and methyl green. Shows mode of formation of mitosome, and of the tail.

FIG. 43. Metamorphosing Spermatid: *Chaetaster longipes.* Platinum chloride 0.3% (12 hours); dahlia and methyl green. Shows mode of formation of the tail.

FIG. 44. Metamorphosing Spermatid nearly transformed into the spermatozoön. *Sphaerechinus granularis.* Sea-water + dahlia and methyl green.

FIG. 45. Metamorphosing Spermatid nearly transformed into the spermatozoön. *Arbacia pustulosa.* Chloride of manganese 10% + dahlia and methyl green.

FIG. 46. Metamorphosing Spermatid: *Echinus microtuberculatus.* Sea-water + dahlia and methyl green.

FIG. 47. Metamorphosing Spermatid nearly transformed into the spermatozoön. *Arbacia pustulosa.* Chloride of manganese 10% + dahlia and methyl green.

FIG. 48. Spermatid nearly transformed into a spermatozoön. *Sphaerechinus granularis.* Sea-water + dahlia and methyl green.

FIG. 49. Spermatid, almost completely transformed *Asterias glacialis.* Osmic vapor. Centrosome and mitosome seem to be rendered more refringent, and are blackened somewhat more than the rest of the cell. The mitosome has not yet become a single mass.

FIG. 50. Spermatid nearly transformed into a spermatozoön. *Arbacia pustulosa.* Chloride of manganese 10% + dahlia and methyl green.

FIG. 51. Mature spermatozoön: *Asterias Forbsii.* Sea-water + dahlia, drawn while it was living. Note the structure of the centrosome (*c*), also compare with centrosome in Figs. 14, 22, 41, 42, 43, 49.

FIG. 52. Mature spermatozoön of *Arbacia pustulosa* after one hour in chloride of manganese 10% + dahlia.

FIG. 53. Mature spermatozoön of *Arbacia pustulosa*, but after a drop of methyl green, aqueous solution was run under the cover-glass. The nucleus has burst and disappeared, leaving only the mitosome and tail visible.

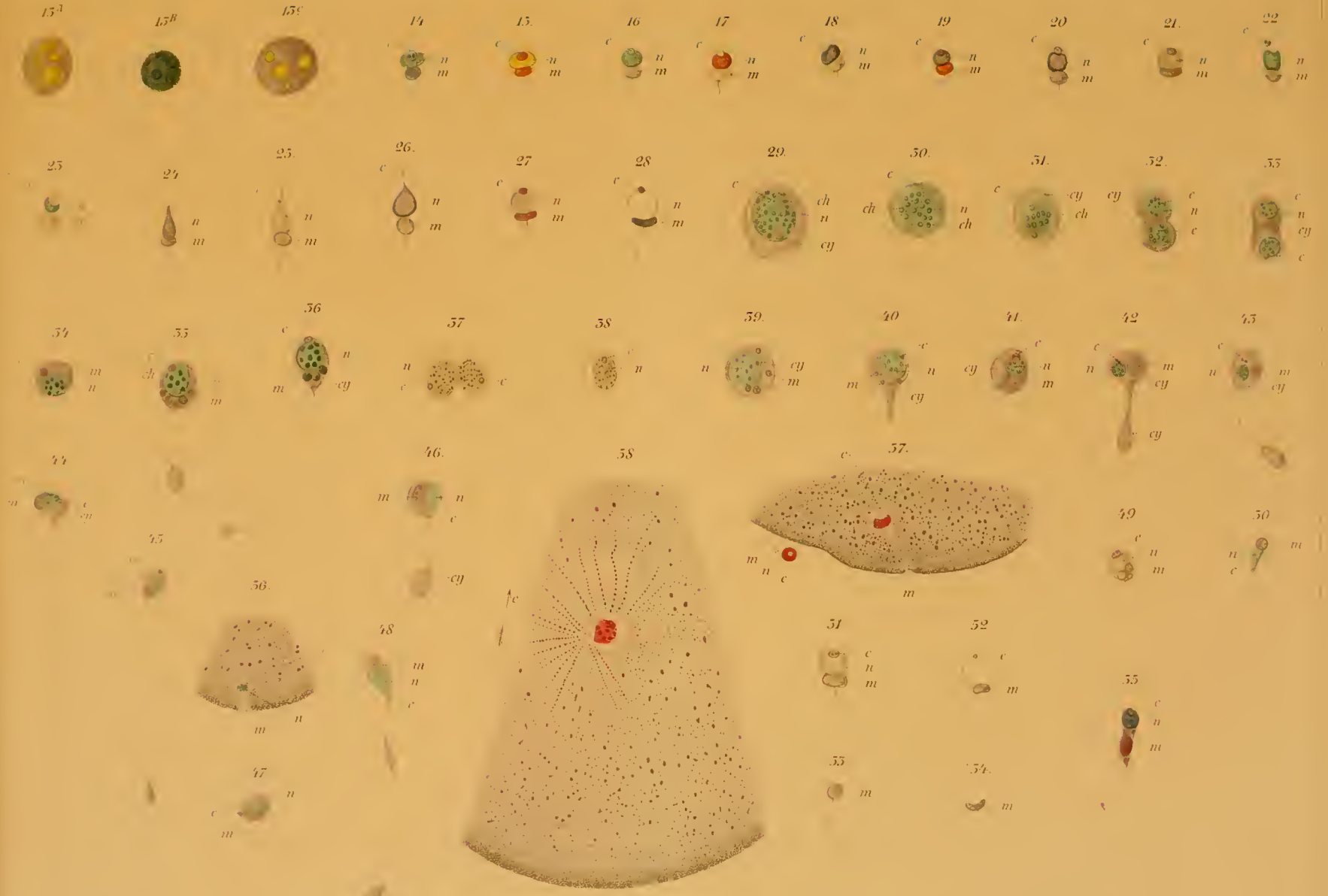
FIG. 54. The same result upon similar treatment of a spermatozoön of an Asteroidea.

FIG. 55. Spermatozoön, very nearly mature. *Chaetaster longipes.* Sea-water + dahlia.

FIG. 56. Spermatozoön of *Asterias glacialis* very soon after entering the cytoplasm of the ovum. Dahlia, methyl green, glycerine preparation. The mitosome is left behind in the periphery of the cytoplasm of the ovum.

FIG. 57. Spermatozoön of *Asterias glacialis*, later. Flemming, section in paraffin. Safranin, section in paraffin.

FIG. 58. Spermatozoön of *Asterias glacialis.* The male pronucleus; the centrosome now lies at the side of the nucleus of the spermatozoön. The radiations are beginning to appear. The arrow shows the direction which the male pronucleus is taking to meet the female pronucleus. The mitosome and tail have been absorbed. Section in paraffin.


13A
13B
13C
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THE SPERMATOGENESIS OF LUMBRICUS.

GARY N. CALKINS.

THE spermatogenesis of the earthworm is characterized, in its external features, by a number of peculiarities which have not yet been brought into relation with the more usual types. The only published work on the subject is by Bloomfield ('80), who gave a fairly accurate account of the external features, drawn chiefly from living specimens or from glycerine preparations, but he failed to make out the internal changes undergone by the developing cells. Bloomfield's principal results may be briefly summarized as follows: (1) The early germ cell is not entirely used in the formation of spermatozoa; a central part remains passive and serves to carry the developing spermatic cells. This central part is called the sperm blastophore and may or may not be nucleated. (2) The sperm blastophores increase by division while in the testis, and disappear, probably by atrophy, after the spermatozoa leave them. (3) The blastophore corresponds to the nucleated supporting cells (Sertoli cells) of the frog and salamander. (4) The large nucleus of the early sperm cell divides many times to form "secondary" nuclei which "stand out around the central mass or blastophore of the generating spheroid with very little protoplasm clothing them." These nuclei become the rod-like heads of the spermatozoa. (5) The protoplasm collects in a small "cap or knob-like mass at the distal end" of the developing cell, and from this grows out the long vibratile tail of the spermatozoön. (This "mass" must be the archoplasm of the spermatid.)

The present investigation was first undertaken in the hope of explaining the significance of the "sperm blastophore" and of identifying the various stages of spermatogenesis in accordance with the usual terminology. It soon became apparent that *Lumbricus* is an especially favorable form for tracing the origin of the various parts of the spermatozoön, and especially the history of the archoplasm, and to this division of the sub-

ject the following paper is mainly devoted. The history of the nuclear elements proved less easy to follow and the results are less satisfactory, although they present certain new points of interest.

The nomenclature adopted is that of LaValette St. George ('78), according to which the three principal stages of the developing cells are designated as (1) "spermatogonia," (2) "spermatocytes," and (3) "spermatids." It is, however, difficult strictly to apply these terms to the Annelida, for the different stages are not confined here to different zones of the testis. In *Lumbricus*, for example, the later stages occur in the seminal vesicles, while the testes contain only spermatogonia. Careful study of the vesicles has failed to demonstrate in them any definite arrangement or order comparable to the "*Wachsthumszone*" or "*Reifungszone*" found in many other forms.

Relative position, therefore, is not a guide to the distinction between spermatogonia, spermatocytes, and spermatids; nor can dimensions of cells be utilized as a means of recognition, for the same stages are represented by quite diverse sizes and forms. The progressive development of the cell is, however, indicated by differences in the make-up and in the number of chromosomes as many observers have shown, and here, therefore, a trustworthy basis of comparison may be found. According to recent investigations the presence of *Vierergruppen* indicates the late stages of "spermatocytes of the first order" or cells where there are one half the normal number of chromosomes and where each chromosome is in four distinct parts. "Spermatocytes of the second order" are recognized both by the half number of chromosomes and by the fact that each chromosome is no longer quadruple but double (*Zweiergruppen*, or double chromosomes). Spermatids are recognized, in their early stages, by the presence of single chromosomes of one half the normal number; in the late stages by the compact and homogeneous chromatin and by the elongating form. The mature spermatozoön consists of head, middle-piece (*Mittelstück*), and tail, parts which are represented in the spermatogonium by nucleus, archoplasm, and a part of the cytoplasm, respectively.

The state of differentiation of the sperm blastophore is a superficial guide to differences in age of the cells, but can be depended upon only to mark the latest stages, where spermatids and blastophores are connected by the merest traces of protoplasm.

I desire to express my gratitude to Professor Wilson for his continued encouragement and advice. Also I take this opportunity to express my indebtedness to the American Association for the Advancement of Science for the use of their Investigators' Room at the Marine Biological Laboratory during the season of 1894.

I. Methods.

My best results have been obtained by killing with Hermann's fluid, a solution consisting of fifteen volumes of platinum chloride one per cent, two volumes of osmic acid two per cent, and one volume of glacial acetic acid. Specimens in various stages of sexual development are selected, and cleaned with filter paper in the usual manner. Segments 9 to 13 inclusive are then cut from the worms and left in the Hermann solution for not more than thirty minutes. By this method sections of the testes and seminal vesicles can be obtained *in situ*. When merely the contents of the seminal vesicles are desired a simple and much more satisfactory method is to cut away from the dorsal side the entire alimentary tract together with the calciferous glands, leaving the large seminal vesicles exposed. The sections may then be stained on the slide. For staining, my best results were gained by the use of Heidenhain's iron haematoxylin, and the safranin, gentian violet, and orange triple stain of Flemming. Good results were also obtained by Mayer's haemacalcium and the Biondi-Ehrlich mixture.

The above methods involve the use of heat and imbedding in paraffine, a process which almost invariably shrinks the tissues of *Lumbricus*. To avoid this difficulty I found it advantageous to use teased specimens. The method is as follows: the large seminal vesicles of sexually mature worms are selected and teased in a watch crystal containing, usually, the killing agent. The teasing is easily accomplished with fine

needles, for the groups of spermatic cells are not attached to the trabeculae of the vesicles. After an exposure of not more than ten minutes in Hermann's fluid the acid is drawn off and the cells washed many times with distilled water, allowing them to settle each time. The cells are then passed successively through fifty, seventy, and ninety per cent, and are finally brought into absolute, alcohol. With a fine pipette they are then transferred to a slide coated with equal parts of egg albumen and glycerine. The alcohol evaporates rapidly, leaving the cells firmly attached to the glass by the fixing substance. The only care necessary is to prevent actual drying of the specimens. After this treatment slides can be handled roughly in the alcohols and stains without detaching the cells. This method offers great advantages over the preceding, and gives, I think, truer pictures. Any stain may be used with these preparations. For archoplasm masses Kleinenberg's haematoxylin gives the best result, but curiously enough this stain does not affect the archoplasm in sections prepared either by the paraffine or celloidin method. Chromosomes are most clearly defined by the use of Flemming's triple stain, while the best differentiation of the cytoplasmic and nuclear elements is obtained by the combined use of iron haematoxylin and orange.

Good results are also obtained by fixation with corrosive sublimate, and with chromic and picric acid solutions. These acids, although unquestionably distorting the cells, distribute the nuclear elements to a certain extent and render them more conspicuous. Their peculiar effect on the archoplasm will be described later.

II. The Blastophore.

The blastophore, considered by Bloomfield ('80, '81) as equivalent to the vertebrate Sertoli cell, is thus briefly described in his paper: "It is in this stage (late spermatogonium) that there is first any indication that as the spermatoblasts are being formed, a slight quantity of protoplasm is being left behind in the center of the generating polyplast, which, as development proceeds, will form the cushion on which the sperm rods may rest. It is best seen in polyplasts which have

been subjected to pressure, when the filament cells or spermatoblasts will be squeezed asunder, but remain connected with the central substance by fine strands of protoplasm. This central mass is the blastophore."

My observations confirm Bloomfield's in regard to the origin of the blastophore, but his description is so meager that a further account will not be superfluous.

These "cushions" with their developing cells (spermatospheres) can best be studied in teased specimens. They are frequently of fantastic shape (Fig. 5), a condition which led Bloomfield to suggest a normal increase by division of the entire blastophore. They become spherical during the later stages of spermatogenesis, especially during the metamorphosis of the spermatid.

A testis of *Lumbricus* in section exhibits numerous ellipsoidal and multinucleate cells (Figs. 1 and 2). The number of nuclei in these cells is inconstant, varying from one or two to as many as twenty, and at this stage they are distributed through all parts of the cell, in the center as well as at the periphery. A study of the various forms shows that the single nucleated cell represents the earliest germ cell, which, by repeated division of its nucleus, gives rise to the multinucleated form. Division figures are frequently seen, and it is a curious fact that in whatever stage of maturation a group may be, the nuclei are all in the same stage of activity at the same time (Figs. 5, 12, and 43).

The multinucleate cells pass from the free edges of the testes into the seminal vesicles, and sections of the latter show that changes in the distribution of the nuclei have taken place. They no longer lie without order in the cell but are arranged around the periphery like the nuclei of an arthropod egg (Fig. 3). The resemblance to the centrolecithal egg is even more striking as development continues, for cytoplasmic cleavage occurs later around each nucleus (Fig. 4), thus differentiating the blastophore from the germ cells. These cleavages deepen until the attachment of the germ cells to the blastophore is reduced to a thin film of cytoplasm. The blastophore remains non-nucleated throughout.

The blastophore, therefore, seems merely an excess of cytoplasm, and evidently is not morphologically the equivalent of a vertebrate Sertoli cell as Bloomfield assumes. The Sertoli cell, according to Watasé ('92), is derived from the primordial germ epithelial cells; according to von Ebner ('88) it is derived from a spermatogonium which ceases to divide and is devoted to the absorption of nutriment in the shape of fats, *etc.*, for the use of the developing germ cells. These cluster about and upon the Sertoli cell, where, like parasites upon a host, they complete their development. The blastophore, on the other hand, is not a cell, for it has no nucleus. It lives as long as the developing germ cells are connected with it and dies when deserted by the spermatozoa. Nor is there any reason to suppose that it provides nutriment for the spermatic cell.

It is conceivable, however, that the vertebrate Sertoli cell was derived from some such structure as the Annelid blastophore. Both originate from the early spermatogonia or perhaps from the earlier reproductive tissue; neither of them forms any part of the mature spermatozoön; the function of the former, however, is clearly defined, while that of the latter is purely conjectural. If, as appears not improbable, the blastophore is merely an excess of cytoplasm, the nuclei of the multinucleate cell upon migrating to the periphery of this cell must have about them that portion of the cytoplasm which is essential to the formation of the mature spermatozoön. If one of the nuclei should remain behind, the result would be, morphologically, a Sertoli cell.

I have been unable to follow the history of the blastophores after they have been deserted by the mature spermatozoa. Their appearance is so altered that we hardly recognize them as the same substance as the cytoplasm of the spermatid. At this time they resemble more closely the protoplasm of protozoa after diffluence. It is probable that their final disappearance is due to atrophy, as suggested by Bloomfield, although many of them serve as food for embryonic parasites (*Monocystis*) which invariably infest the seminal vesicles of *Lumbricus*.

III. Phenomena of Reduction.

As previously stated, my results regarding the reducing divisions are not wholly satisfactory, owing to the smallness of the cells and to their lack of consecutive arrangement in the testis and vesicles.

A. *Spermatogonia.*

The spermatogonia are represented in the testes by the multinucleate cells (Fig. 2). The nuclei are small, yet larger than those of the epithelial cells from which they arose, and as a rule have no nucleoli (Figs. 2 and 3). The chromatin is distinct but not abundant, nor is there any characteristic form. In the cytoplasm by the side of each nucleus is a large and rather indefinite faintly staining body (see Section V). As the nuclei prepare for division, the *Knäuel* or spirem stage is less conspicuous than in the later sperm cells, its filaments being fine and indefinite. In full karyokinesis neither asters nor astral rays can be seen. Picric acid preparations show small dots, the centrosomes, or archoplasm masses at the poles, and spindle fibres can be traced directly into the chromosomes (Fig. 8); the nuclear plate contains thirty-two small and apparently spherical chromosomes (Figs. 7 and 8).

B. *Spermatocytes of the First Order.*

Spermatocytes of the first order cannot be distinguished by their external appearance. The nuclei have migrated, however, to the periphery of the cell (Fig. 3). I am unable to assert whether karyokinetic divisions of these nuclei intervene between the divisions occurring in the testis and the migration of the nuclei to the periphery of the cells in the seminal vesicles. Such, however, is probably not the case, because of the scarcity of karyokinetic figures in the testis, and because of the average number of nuclei in the multinucleate cell. It seems probable, therefore, that the final division in the testis gives rise to spermatocytes of the first order. Each of these

contains thirty-two chromosomes (Fig. 9). The nuclei enlarge after this final division and the entire spermatosphere increases in size (compare Figs. 4, 6, and 12). The archoplasm also is large and distinct (Fig. 4, *A*).

The resting nuclei of this stage are large and round and exhibit well marked nucleoli. It has been frequently stated that, in the case of developing spermatic cells, the nucleolus is thrown into the cytoplasm; this certainly does occur in *Lumbricus* (Figs. 44 and 46), but from the nature of these preparations (these results were seen in only one series of sections) I have not the least hesitancy in asserting that the phenomenon is here an artefact.

After a period of rest (Fig. 10) during which the cytoplasmic cleavages deepen, the nuclei begin their period of greatest activity. The chromatin collects at one portion of the nucleus in a rather small lump (Fig. 11) which afterwards expands and becomes mesh-like, while fine offshoots of chromatin soon appear (Fig. 12). The chromatin elements grow during this period and a thick spirem is formed, nearly filling the nuclear space. The thread is ragged at first (Fig. 12), but later becomes apparently smooth (Figs. 12 and 13). In favorable preparations the spirem is seen to be split longitudinally, and is therefore double (Fig. 12, *D*). The cell elements are much too small and the spirem much too twisted and interlooped to tell whether it is one long piece. In one case, however (Fig. 13), I was able to see that at most there were only two pieces. Later the spirem becomes transversely segmented into many rod-like bodies (Figs. 14 and 15), which at first appear to be without order, but in later stages are found to be definite both in number and in structure. It is then seen that there are thirty-two of these bodies and that each is double (Fig. 16). From them the "*Vierergruppen*" are formed by a process differing widely from the method usually described. The thirty-two double chromosomes unite two by two (Figs. 17–20), and are finally arranged in sixteen quadruple groups (Fig. 21). In some cells four quadrivalent groups were seen, together with twenty-four double chromosomes; in others twelve quadrivalent groups and eight double forms were seen (Fig. 19).

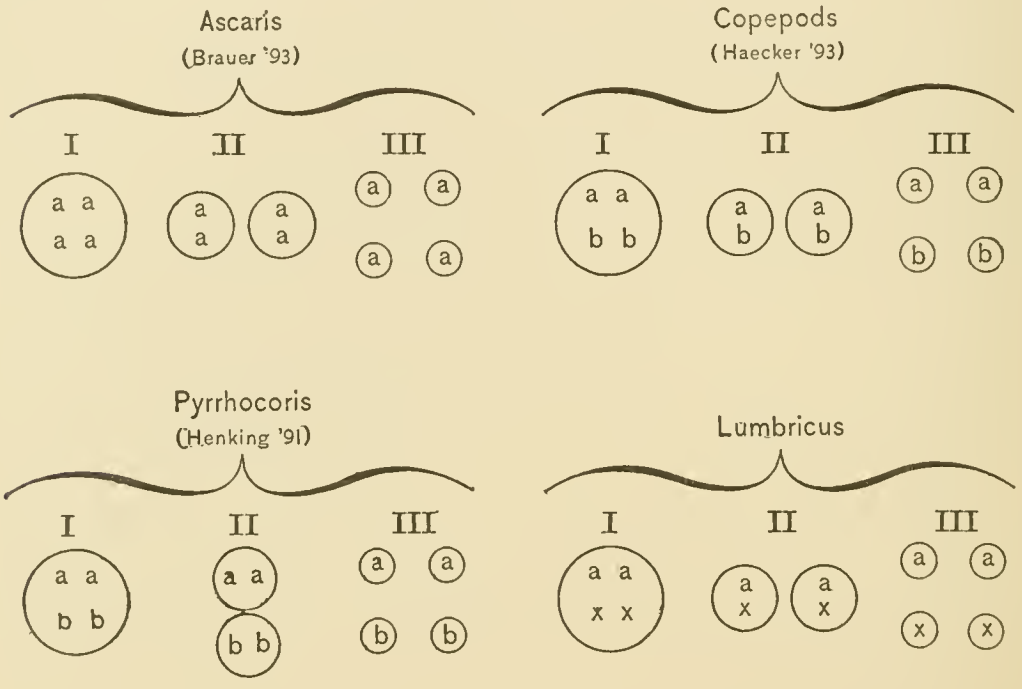
The further history of this stage is as follows: during the ensuing cell division (Figs. 22–27) the "*Vierergruppen*" are halved, sixteen double chromosomes going to each daughter cell. Whether this is a reducing division in the Weismann sense cannot be ascertained. It is interesting to note that in no case is the axis of the spindle directed towards the center of the spermatosphere (Figs. 5 and 43). The division planes are radial and the daughter cells thus remain attached to the blastophore.

The reduction in the number of chromosomes has now taken place, not during mitosis, however, but during the antecedent period and through the activity of the chromosomes themselves. The process thus corroborates the view advocated by Boveri ('89) and sustained by Brauer ('93) and others, that the chromatin particles have power to arrange themselves. The formation of the "*Vierergruppen*," however, differs from that which takes place in *Ascaris*.

Two main conceptions of the "*Vierergruppen*" and their mode of origin have been current. One, as advanced by Brauer ('93), is that each quadruple chromosome originates by a double splitting of each chromatic element, *i.e.*, by two longitudinal divisions of each chromosome. The other, advanced as a theory by Weismann, and since demonstrated by Ishikawa ('91), Haecker ('93), vom Rath ('92), Henking ('91), and most ably by Rückert ('93, '94), is that the "*Vierergruppen*" originate by two divisions of the chromosomes, the first being longitudinal, the second transverse.

According to my conception the origin of the "*Vierergruppen*" in *Lumbricus* is quite different from either of the above. It is as follows: there is indeed a horizontal division (Fig. 12) (shown by the double spirem), and later a transverse division, but this gives thirty-two double instead of sixteen quadruple chromosomes; the "*Vierergruppen*," or quadruple chromosomes, are formed later by union of these double chromosomes two by two. In the forms examined by Rückert sixteen double chromosomes are formed; these are arranged in a nuclear plate and transverse division takes place while in this position, thus forming the "*Vierergruppen*."

These differences can best be seen, perhaps, by the aid of diagrams. If *a*, *b*, *c*, *d*, *etc.*, represent consecutive chromatin elements in the monospirem, the following series can be obtained, in which I indicates the composition of the *Vierergruppen*, II the first division, and III the second division:



According to this schematic arrangement, the *Vierergruppen*, which, in most cases, consist of two contiguous elements (*a* and *b*) doubled, in *Lumbricus* consist of the element *a* doubled, and united with any other element, *x*, also doubled. In this case the end result is the same as in *Pyrrhocoris* or in the copepods, but the method of arriving at it is different.

The process, as in *Lumbricus*, is carried a step further in *Caloptenus*, where, according to Wilcox ('95), there is no preliminary doubling of the spirem; and, since there is here a union of the spirem segments as in *Lumbricus*, the elements of the *Vierergruppen* would be indicated thus: $\begin{matrix} a & b \\ x & y \end{matrix}$, *x* and *y* representing any other chromatin segment than *a* and *b*.

The results obtained in *Lumbricus* may, therefore, serve as a link in the chain, the extremes of which are represented by the views of Brauer and Wilcox.

C. *Spermatocytes of the Second Order.*

In spermatocytes of the second order there are but sixteen double chromosomes — one-half as many as in the spermatogonia. After their division the daughter cells, or spermatids, contain sixteen single chromosomes (Figs. 28 and 29).

The following distinctions may now be drawn between spermatocytes of the first and second orders: in the first order occurs reduction in *number* of the chromosomes due to chromatic activity; this is followed by a reduction in *quantity* of chromatin through karyokinetic division; in the second order the chromosomes are passive, while reduction in mass occurs through karyokinetic division.

D. *Spermatids.*

The spermatid nucleus is the result of the double reducing division. It contains only half as many chromosomes as in the spermatogonium.

After division of the spermatocytes of the second order the chromatin gives rise to a reticulated mass which gradually becomes more and more compact and homogeneous (Figs. 30–33). The spermatid is a small round cell closely attached to the outside of the blastophore (Fig. 6). The archoplasm lies at the extremity furthest removed from the blastophore and is closely applied like a cap to the nuclear membrane (Fig. 35), or lies like an independent cytoplasmic sphere between the membrane and the extremity of the cell (Fig. 6). The cytoplasm has no other distinctive feature; it is small in amount and apparently only sufficient to cover a comparatively large nucleus. There is no indication of granules, which are so characteristic of the blastophore at this stage (Fig. 36).

The mature spermatozoön is developed from the spermatid by a simple metamorphosis. The nucleus elongates, and the distal extremity becomes drawn out to form the tail.

The first indication of the spermatid metamorphosis is loss of reticulation in the nucleus (Figs. 31, 32). The chromatin becomes dense and homogeneous, forming at first a rounded mass like a ball lying within the nucleus (Fig. 33), but later becoming

cylindrical, with rounded ends (Figs. 35 and 36). At the same time the beginning of the tail appears as a minute pointed prominence of cytoplasm immediately above the archoplasm at the distal extremity of the cell (Fig. 6). As the elongation continues the chromatin portion becomes longer and thinner. In chromic acid preparations it still retains its reticulated appearance (Fig. 36), but after Hermann's fluid it is more dense and compact (Fig. 35), and ends distally in the growing tail filament. The archoplasm, which in some cases is drawn out distally, lies in the triangular area at the base of the tail, and, at a later stage, a large vesicle is formed about it (Fig. 37, *V*) which is typical of the spermatid at this time. The further history is briefly as follows: the nucleus and tail elongate still more; the vesicle disappears by elongation of the archoplasm mass, and the head of the spermatozoön finally assumes the shape of a long rod with a much longer filamentous tail.

The cytoplasm is apparently stretched to its utmost, for only the most careful observations on favorable preparations will reveal it lying as a mere line around the nucleus and middle-piece (Fig. 37). The young spermatozoön now consists of a filament composed of three parts. First: the nucleus or head, which is directed towards and seems to be attached to the blastophore, but which is in reality separated from it by a small amount of cytoplasm (Figs. 37 and 38 *s*). Second: the middle-piece, which appears as a direct continuation of the nucleus so that it can be distinguished only after differential staining (Figs. 38 and 47 *m*). Third: the tail or flagellum, which is much longer than the nucleus and much finer. The small amount of protoplasm between the head of the spermatozoön and the blastophore becomes drawn out into a sharp spur after the cell breaks away (Fig. 47 *s*), and this, according to the observations of Foot ('94), acts as a boring-point with which the spermatozoön pierces the egg.

IV. ARCHOPLASM.

The spermatogenesis of *Lumbricus* offers exceptional advantages for the study of archoplasm, which forms a conspicuous

body in the developing cells, and can be readily demonstrated by the use of most stains.

The position of the archoplasm in the spermatozoa of different animals is interesting because of its bearings on fertilization of the egg and origin of the male attraction center within the egg. The origin of this body within the egg has been observed in only a few cases. Fick ('93) followed its history in the fertilization of the *Axolotl* egg and found that its *Anlage* is the middle-piece of the spermatozoön. Foot ('94) has recently examined the fertilization and maturation processes in *Allolobophora foetida* (*Lumbricus foetida*), and asserts that the attraction center seems to arise from the middle-piece of the spermatozoön. Fol ('91) asserted that in the fertilization of the echinoderm egg, the male attraction sphere originates from the anterior tip of the spermatozoön, but Wilson ('95) has clearly shown that Fol's interpretation of the fertilization process in echinoderms was erroneous. Besides demonstrating the absence of Fol's "Quadrille of the Centers" he shows that the attraction sphere in the fertilized eggs of *Toxopneustes variegatus* is derived from the middle-piece of the spermatozoön, which rotates through an angle of 180 degrees after entering the egg. Mathews ('95) has observed the same rotation in the fertilized eggs of *Asterias* and *Arbacia*. In all of these cases, therefore, where the process of fertilization has been carefully followed, the "sperm center" originates from the middle-piece of the spermatozoön.

What now is the relation between the archoplasm (*i.e.*, sperm center) in the middle-piece of the spermatozoön, and the archoplasm of the early germ cells? Is the "sperm center" composed of the same substance as the archoplasm of the spermatocytes and spermatid?

There are unfortunately too few cases where the fertilization processes have been carefully followed out to enable us to decide this question, and in the cases cited above the results of different investigators are, in a measure, contradictory, while the results obtained by other observers apparently cohere. For example, in the *spermatogenesis* of the echinoderms, Field ('93) has followed the centrosome from the early spermatic

cells to a position at the extreme tip of the spermatozoön, whereas in the *fertilization* of the echinoderm egg Wilson ('95) and Mathews ('95) have shown beyond question that the sperm center originates from the middle-piece. In the fertilization of the egg, and in the spermatogenesis of *Lumbricus*, however, the results of different observers agree. In the fertilization of the *Lumbricus* egg Foot ('94) has shown that the sperm center probably arises from the middle-piece, and in the spermatogenesis of *Lumbricus* I have shown that the archoplasm is a continuous element of the cell and forms ultimately the middle-piece of the spermatozoön ('94).

Many other investigators besides Field have followed the course of the centrosome from the early sperm cells to the anterior end of the spermatozoön, but in most cases the fertilization of the eggs has not been studied. Flemming ('88) in the spermatogenesis of the salamander was in doubt as to the origin of the extreme tip of the spermatozoön, but thought that the middle-piece originated from the chromatin. Platner ('85, '89) showed that the extreme tip of the spermatozoön in butterflies and in some pulmonates (*Helix pomatia* and *Limax agrestis*) is formed from the centrosome. Benda ('91) showed that in the spermatozoa of mammals, birds, and reptiles, the "*archiplasma*" forms the *Spitzenknopf* and *Kopfkappe*, and Moore ('94) asserted that in rat spermatogenesis a portion of the archoplasm (archosome) forms the anterior tip of the spermatozoön, but that the place of the *Mittelstück* "is apparently occupied by the spermatid centrosomes and an *intermediär Körperchen*"

A possible explanation of some of these differences may be that in many cases the "*Nebenkern*" is confounded with the archoplasm, and that the extreme tip of the spermatozoön is formed from this or from a portion of it, instead of from the centrosome and archoplasm. Henking ('91) showed that in the spermatozoön of *Pyrrhocoris apterus* the tip of the spermatozoön is formed from a part (*Mitosoma*) of the *Nebenkern*.

I have found two peculiarities in the archoplasm of *Lumbricus* due to treatment of the preparations. First in prepara-

tions which have been imbedded either in paraffine or in celloidin, the archoplasm does not stain so readily as in teased specimens. Second, different killing agents affect the archoplasm in different ways. After Hermann's fluid it is large and has a more or less reticulated appearance. After picric or chromic acid or corrosive sublimate it is much contracted, and in full karyokinesis appears as a minute and homogeneous dot at the spindle pole. The differences in appearance following the use of these various reagents is quite remarkable and cannot be attributed to individual variation in the several cells.

I have been unable to trace the early history of the archoplasm in spermatogonia, but in the fully developed spindle it may be seen at the two poles (Fig. 8, a specimen killed with picric acid). There is here no indication of radiating cytoplasmic fibres (astral rays).

The archoplasm is a much more favorable object for study in spermatocytes of the first order, since it may be observed in teased specimens which can be more easily stained, whereas in spermatogonia the cells are necessarily studied from sections. In teased preparations from the vesicles the spermatocyte archoplasm can be best demonstrated by staining with Kleinenberg's haematoxylin. An exposure of fifteen minutes is sufficient to turn them a dark blue, the other structures of the cell remaining quite unstained. The archoplasm masses are then so distinct that their history can be easily followed, although preparations which show these changes to the best advantage do not indicate the corresponding changes of the chromatin, and the various parts of the cell must, therefore, be studied independently.

The normal position of the archoplasm is at the distal extremity of the nucleus, although I have frequently seen it at the opposite extremity as well as at all intermediate positions. In some cases it is elongated and flattened and lies closely applied to the exterior of the nuclear membrane. In other cases it is divided into finger-like processes and loops, closely resembling certain stages of the so-called *Nebenkern* as figured and described by Platner ('85) in the spermatic cells of *Limax ag.* and *Helix pomatia*. Again it is in the form of a sphere;

— but in all forms it is one and the same substance. It divides at the beginning of nuclear activity (Figs. 12, 39 and 45), and, if it occupies a distal position, each half travels around the nucleus through an arc of about 90 degrees (Figs. 39, 40, 41, 42). This makes the axis of the spindle tangential to the periphery of the central sphere, and the subsequent division plane passes radially through nucleus and cytoplasm in such a manner that the resulting daughter cells remain attached to the blastophore. The spindle fibres are formed directly from the archoplasm masses (Fig. 45). In Hermann fluid preparations (teased) the archoplasm can be considered as nearly true to life as is possible with this material. Such is the condition represented in Fig. 43, one of a large number of similar preparations; the archoplasm extends partly up the spindle fibres, and the latter appear taut, as though they had been pulled from the main mass. In some cases the archoplasm extends up the spindle fibres as far as the chromosomes (Fig. 43). After division of the chromosomes the spindle fibres seem to be in part drawn back into the archoplasmic substance (Figs. 5, and 43 *A*), although I do not mean to assert that all of the spindle fibres are withdrawn in this manner.

After the reconstruction of the nucleus the archoplasm lies upon the nuclear membrane like a closely fitting cap. It soon becomes spherical, preparatory to redivision, but meanwhile it wanders from its position at the side of the cell to the distal extremity.

I have been unable to follow the division of the archoplasm in spermatocytes of the second order, although it is distinctly seen at each pole of the karyokinetic spindle (Fig. 29). From here it passes directly into the spermatid archoplasm, which appears very distinctly, and is much more susceptible to stains than at any previous period. It is the same in size as at any antecedent resting-nuclear stage (Figs. 4, 12, and 35), and the idea that it is of nuclear origin cannot for a moment be sustained. It lies upon the distal part of the nuclear membrane, and since after karyokinesis it lay at the side of the cell, its position here can be explained only by the supposition that it has moved through an arc of 90 degrees, or that the nucleus

revolves so as to bring the archoplasm into this position. The former is the more reasonable hypothesis.

The further fate of the archoplasm may be described as follows : the spermatozoön is formed from the spermatid by elongation in the direction of its radial axis. The nucleus of the spermatid, which is here compact and homogeneous, elongates, and a tail forms at the distal extremity of the cell (Fig. 6). This elongation of the nucleus continues until the spermatid becomes a long columnar cell, with a filament growing out from one end, the other end remaining attached to the blastophore. The archoplasm mass now lies in a vesicle between the tail and the nucleus (Fig. 37). The nucleus and tail elongate still more ; the vesicle disappears by elongation of the archoplasm mass, and the spermatozoön finally assumes the shape of a long rod with a much longer filamentous tail (Fig. 38). This rod consists of middle-piece and nucleus which can be differentiated only by careful staining. The archoplasm mass of the early sperm cells and the middle-piece of the spermatozoön of *Lumbricus* are, therefore, one and the same substance.

The action of acids upon the archoplasm mass deserves further notice. Why should picric acid, for example, reduce the archoplasm from such a structure as that seen in Fig. 43 to that in Fig. 8 in active nuclei, or from such a structure as that seen in Fig. 4 to that in Fig. 41 in resting periods? The condensation here may give some new light on the nature of centrosome structure. It lends support to the view of recent cytologists, that the centrosome is an uncertain and indefinite element of the cell and composed of granules in a greater or less degree of density.

If, as these observers suppose, the centrosome is but an aggregation of protoplasmic granules, it is easy to conceive that in the archoplasm of *Lumbricus*, these granules are not tightly packed, and that a favorable fixing agent will preserve them in this condition. Such a result is given by the use of Hermann's fluid on teased specimens. If a killing agent is used which does not act so quickly as the platino-osmic-acetic mixture, it is conceivable that a condensation of the granules

may take place. Certainly in chromic and picric acid preparations the archoplasm masses are greatly reduced, and stain much more intensely with miscellaneous stains than in other cases.

An accessory body (*Nebenkern*) is often found in the developing germ cells of *Lumbricus*, usually in the spermatocytes of the first order. This body also appears in the spermatic cells of a great variety of forms where it is often mistaken for archoplasm. It was discovered by LaValette St. George ('67), and has been repeatedly described by subsequent observers. Bütschli ('71) gave to this body, which he found in the spermatic cells of insects and crustacea, the name *Nebenkern*, a name which has clung to it ever since.

The term *Nebenkern* ("accessory nucleus") is unfortunate, for in it there is nothing to designate any peculiarity or characteristic of the body in question, and it might as well be applied to any unusual structure of the cell. Such various application has, indeed, been made. O. Hertwig ('75) used the term *Nebenkern* to designate the micronucleus of the ciliates. Nussbaum ('82) applied the same name to bodies constricted off from the nucleus in cells of the hepato-pancreas, and Blochmann ('84) gave the name "*Nebenkern*" to the "yolk-nucleus," a body found in the early stages of the developing egg cells. Many later writers have used the term in a more or less undefined manner, until to-day the word *Nebenkern* has no especial significance.

In all of these cases, with the exception of Hertwig's application of the term to infusoria, the name *Nebenkern* is unsuitable and non-descriptive, for the structure so designated is not a nucleus, although perhaps nuclear in origin. In the case of the infusoria the structure called *Nebenkern* is much better described by the term "micronucleus" which is now generally adopted. The other bodies in the cell to which the name *Nebenkern* is applied, notably in spermatic cells, in egg cells, and in the glandular cells of the hepato-pancreas, are not homologous with each other; and the same term therefore should not be used to designate them. In the spermatic cells LaValette ('86), Platner ('86, '89) and Henking ('91) showed that

the *Nebenkern* originates as the remnant of the interzonal fibres of the karyokinetic spindle, and that it is, therefore, archoplasmic in origin. In pancreas cells, Nussbaum ('82) showed that the *Nebenkern* is derived by a budding off from the nucleus and is therefore of nuclear origin. The so-called *Nebenkern* in the egg cell originates in the same manner apparently as in the pancreas cells. Valaoritis ('82) described this body as originating by metamorphosis of the germinal vesicle ; Van Bambeke ('93) claimed that it arises by direct transportation of the chromosomes ; and in a recent preliminary paper I ('95) have shown that this body (which Carus in 1850 called the "yolk-nucleus" (Dotterkern) originates from the chromatin network in the nucleus. Here also the so-called *Nebenkern* of Blochmann is nuclear in origin. It is apparent therefore that these bodies which differ so greatly in their origin should not have the same name. In the egg cell the so-called *Nebenkern* is sufficiently well described by the term "*Dotterkern*" or "yolk-nucleus," and a more satisfactory name can be found for the so-called *Nebenkern* of the pancreas.

The word *Nebenkern*, if used at all, should be applied to the element of the spermatic cell as originally proposed by Bütschli ('71), and I shall adhere to this use of the term in the present paper.

A *Nebenkern*, then, can be described as an element of the early spermatic cell originating from the interzonal fibres (*Verbindungsfäsern*) of the karyokinetic figure. It must, therefore, be archoplasmic in origin, for the *Verbindungsfäsern* are derived from the archoplasm. Its function, however, as archoplasm, is lost.

That there is need of such an accurate definition of the term *Nebenkern* in spermatic cells is well shown from the confusion caused by its rather loose application in many instances. For example, Platner ('85, '89) in his several papers on spermatogenesis uses the term *Nebenkern* as the equivalent to Boveri's ('84) *Archoplasma*. This body as described by him, however, seems to be equivalent to both archoplasm and *Nebenkern*, for according to Platner's observations it consists of two parts, both coming from the archoplasmic spindle. These are first, a

central part, derived from the centrosome and which ultimately forms the tip of the spermatozoön, and second, the *Mitosoma*, derived from the equatorial spindle fibres (*Verbindungsfäsern*) which ultimately form the membrane about the axial filament of the tail. The tip of the spermatozoön, therefore, is formed from the *Nebenkern*. Moore ('94) confuses the two terms and calls archoplasm a body originating from the spindle fibres after the disappearance of the spindle. Henking ('91) uses the term *Nebenkern* in the sense of Bütschli, but restricts it to the body formed by the peripheral spindle fibres, while the central spindle forms what he calls the *Mitosoma*. The Mitosoma in *Pyrrhocoris apterus* forms the tip of the spermatozoön, and the *Nebenkern* a caudal membrane. Hermann ('91) also gives to the *Nebenkern* the significance of archoplasm, while Balbiani ('93) considers archoplasm, *Nebenkern*, and yolk-nucleus homologous bodies. Other spermatologists give various meanings to the terms archoplasm and *Nebenkern*, making it difficult to determine whether a certain element in a spermatozoön (as the tip, for example) comes from the centrosome and archoplasm or from the *Verbindungsfäsern* of the karyokinetic spindle.

The *Nebenkern* in *Lumbricus* usually lies at the extremity of the cell opposite the archoplasm, and in some cases it persists even during the process of karyokinesis (Fig. 45).

The presence of archoplasm and *Nebenkern* in the same cell in *Lumbricus* throws some light on the distinction between these bodies. These differences can be tabulated as follows: (1) the *Nebenkern* originates as the remnant of the interzonal fibres, while the archoplasm is a constant element of the cell; (2) the archoplasm plays a certain definite rôle, while the *Nebenkern* has no apparent function; (3) the archoplasm divides and behaves during karyokinesis in the same manner as a so-called centrosome and finally forms the middle-piece of the mature spermatozoön, while the *Nebenkern* is passive and disappears after a seemingly useless existence. These distinctions are not true, however, for all forms. In many cases the *Nebenkern* has an important function, such as the formation of enveloping membranes in the salamander spermatozoa.

The archoplasm as described above is apparently equivalent to the central body (centrosome) and its archoplasm. There is no indication that it is of the nature of a *Nebenkern.* According to Moore ('94) in the spermatogenesis of the rat, the "archoplasm" originates from the spindle fibres of the preceding karyokinesis, probably from the interzonal fibres, or, to quote his own words: "Accordingly in the spermatogenesis of the rat, the sphere seems to be divided into two parts, one made up of nothing but the residual spindle fibres (archoplasm), and another containing nothing but the centrosomes." Again: "The centrosomes divaricate and assume their usual position at the poles of the growing spindle, while the archoplasm remains an inactive structure in the body of the cell." At another place he says: "The archoplasm is reabsorbed into the cytoplasm." In this account of the archoplasm he is simply describing the *Nebenkern* as defined above; its origin from the spindle fibres, its lack of connection with the centrosome, its final absorption into the cytoplasm and its inactivity in the cell, — all these are phenomena of the *Nebenkern* and not of an active cytoplasmic organ like the archoplasm of *Lumbricus.* It is not improbable that the same mistake is made by others, and it may be found that the so-called "centrosome" at the tip of the spermatozoön in many forms is in reality a portion of the *Nebenkern* as Henking has described in *Pyrrhocoris apterus.*

Summary.

The results of my investigation on the spermatogenesis of *Lumbricus* may be summarized as follows:

1st. A multinucleate cell is formed in the testis. This represents a group of the earliest spermatic cells or spermatogonia. Each spermatogonium gives rise to several spermatozoa.

2d. The nuclei arrange themselves around the periphery of the multinucleate cell. Cytoplasmic cleavages then ensue between the nuclei, as in a centrolecithal egg. These cleavages deepen until the nuclei are separated from the central mass of the cytoplasm by mere filaments.

3d. The residual mass of cytoplasm thus formed — the blastophore — is not nucleated and cannot be compared with a Sertoli cell in function, form, or mode of origin. It finally disappears. The blastophore furnishes perhaps the chief source of food supply for the parasites — *Monocystis* — which live in the seminal vesicles. A possible explanation of the function of the blastophore is that it represents superfluous nutritive cytoplasm, the vital protoplasm having gathered around the nuclei.

4th. There is a reducing division in the number of chromosomes and in the quantity of chromatin. In the spermatogonium thirty-two single chromosomes divide, giving thirty-two to each spermatocyte of the first order. During the succeeding resting stage the chromatin substance, nucleus, and entire spermatosphere become enlarged. The chromatin emerges as a double skein. It divides by transverse division into thirty-two double chromosomes, and these unite two by two to form sixteen quadruple chromosomes. Reduction in number thus takes place without the aid of karyokinesis. In spermatocytes of the second order the nuclei contain sixteen double chromosomes. In the succeeding division these double chromosomes divide, and spermatids result, each with sixteen single chromosomes.

5th. Metamorphosis of the spermatid takes place independently of external conditions. The nucleus or head is formed from the entire chromatin. *The archoplasm of the spermatid forms the middle-piece of the spermatozoön.*

6th. The archoplasm mass persists throughout every change of the cell. In the preparatory stages of cell division it divides to form the poles of the karyokinetic spindle, as well as the spindle fibres themselves. All evidence points to the conclusion that, during the anaphase, the spindle fibres are partly withdrawn into the archoplasm. The interzonal fibres probably form the *Nebenkern*, which lies quiescent within the cell and finally disappears.

7th. The archoplasm mass is affected by the method of treatment. After Hermann's fluid it remains large and conspicuous, both at the spindle poles and in the resting cells.

After chromic or picric acid it is small, more dense, and stains more deeply.

8th. There is a distinct difference between a *Nebenkern* and the archoplasm. The latter gives rise to spindle fibres, retracts parts of them into its substance and finally becomes the middle-piece of the mature spermatozoön. The *Nebenkern*, on the other hand, is derived from interzonal fibres and disappears after an apparently useless existence.

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DESCRIPTION OF PLATES.

All figures are magnified about 1,600 diameters, except Fig. 1, Plate XIX. Figs. 1–6, 9–33, 35, 37, 38, 43, 43A, 45, and 47 are from preparations fixed with Hermann's fluid; Figs. 7 and 8 with picric acid; Figs. 34, 36, 39–42, 44, and 46 are from preparations fixed with chromic acid.

All figures save 1, 7, 8, 44, and 46 are from teased preparations; the others from sections.

In all figures the following letters have the same meaning:

A. Archoplasm.
B. Blastophore.
C. Cytoplasm.
D. Double spirem (Fig. 12).
F. Four-group (Vierergruppen).
M. Middle-piece.
N. Nucleus.
Neb. Nebenkern.
Nuc. Nucleolus.
S. Spur.
T. Tail.
V. Vacuole.

EXPLANATION OF PLATE XVII.

FIG. 1. A section through the testis, showing position of the latter in relation to the dissepiment. There is no distinct membrane about the testis as there is in the case of the ovary. The nuclei at the extremity of the testis are arranged in groups of varying size. Each group consists of spermatogonia, and each group is one multinucleate cell.

FIG. 2. A multinucleate cell in the testis, showing the general distribution of the nuclei throughout the cell. Optical section.

FIG. 3. Multinucleate cell in a seminal vesicle, showing a peripheral arrangement of the nuclei before the cytoplasmic cleavages have taken place. Optical section.

FIG. 4. A multinucleate cell in the seminal vesicles, showing the beginning of cytoplasmic cleavage very similar to the cleavage of a centrolecithal egg. The interior cytoplasm is finely granular. At the extremity of the nucleus is a large mass of archoplasm which is characteristic of the resting stage.

FIG. 5. An eccentric spermatosphere, showing distortion of the blastophore, increase of spermatocytes of the first order, anaphase of karyokinesis, reconstruction of archoplasm at spindle poles, and beginning of cytoplasmic cleavage with traces of interzonal fibres in some cells.

FIG. 6. A spermatosphere in a late stage, showing elongation of spermatids with beginning of tail formation at the free end of the cell. The archoplasm is at the distal extremity of the homogeneous and compact nucleus.

FIG. 7. A spermatogonium in the testis from a multinucleate cell, showing the nuclear plate with 32 apparently single chromosomes.

FIG. 8. A spermatogonium from the testis in full karyokinesis, showing 24 of the 32 chromosomes and the archoplasm reduced by the picric acid to a mere dot at the poles.


1.
2.
3.
C
C
4.
A
B
N
A
5.
A
A
B
A
A
6.
7.
T
A
N
B
8.

EXPLANATION OF PLATE XVIII.

FIG. 9. Spermatogonium in the anaphase of karyokinesis; chromosomes of the daughter nuclei single and 32 in number (only 19 and 21 are shown in the figure).

FIG. 10. Resting nucleus of spermatocyte of the first order, showing nucleolus and the reticulated chromatin.

FIG. 11. Spermatocyte with the chromatin in a lump at one part of the cell; first phase after the resting period.

FIG. 12. Spermatocyte of the first order. The cells of spermatosphere show different stages in the formation of the Knäuel or spirem. At D the spirem is double. The archoplasm is in different stages of division.

FIG. 13. Spermatocyte of the first order, showing double spirem in at most one or two pieces.

FIG. 14. Spermatocyte of the first order, showing the spirem breaking into parts.

FIG. 15. Spermatocyte of the first order, showing formation of double chromosomes and the presence of Nebenkern.

FIG. 16. Spermatocyte of the first order, showing 32 double chromosomes.

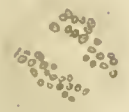
FIGS. 17, 18, 19, 20, and 21, spermatocytes of the first order, showing different stages in the formation of the four-groups, or Vierergruppen.

FIGS. 22, 23, 24, 25, 26, and 27. Different stages in karyokinetic division of the spermatocyte of the first order.

FIGS. 28, 29, and 30. Different aspects of the spindle of spermatocytes of the second order. Fig. 30 represents the nuclear plate of a spermatocyte of the second order, showing 16 double chromosomes.

FIGS. 31, 32, and 33. Spermatids, showing different stages in the concentration of the chromatin.

9.



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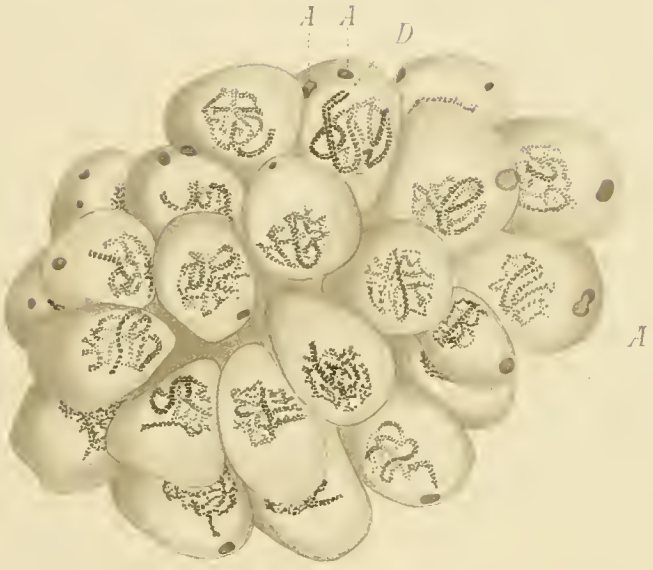
17.

12.



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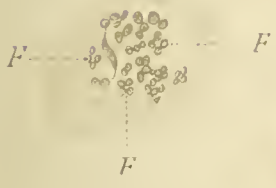
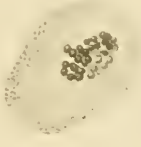


19.

21.

20.

23.



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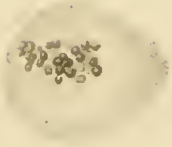
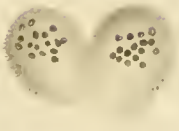


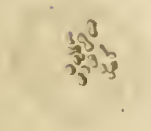
24.

31.



27.

26.



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33.

EXPLANATION OF PLATE XIX.

FIG. 34. Spermatid, showing reticulated nucleus and minute archoplasm mass due to the killing agent.

FIG. 35. Spermatid highly magnified, showing homogeneous nucleus becoming drawn out, tail forming from cytoplasm, and with a clear space around the chromatin. Archoplasm clear and distinct.

FIG. 36. Spermatids on blastophore, chromatin not homogeneous, archoplasm minute (like a centrosome), cytoplasm more or less distorted, all due to the killing agent.

FIG. 37. Spermatid much elongated and detached from the blastophore, showing homogeneous nucleus with a comparatively large vesicle at the extremity which contains the archoplasm mass.

FIG. 38. Spermatid. The vesicle has here disappeared; the archoplasm now connects the tail and nucleus and is the middle-piece.

FIGS. 39, 40, 41, and 42. Spermatocytes of the first order, showing different stages in the history of the archoplasm.

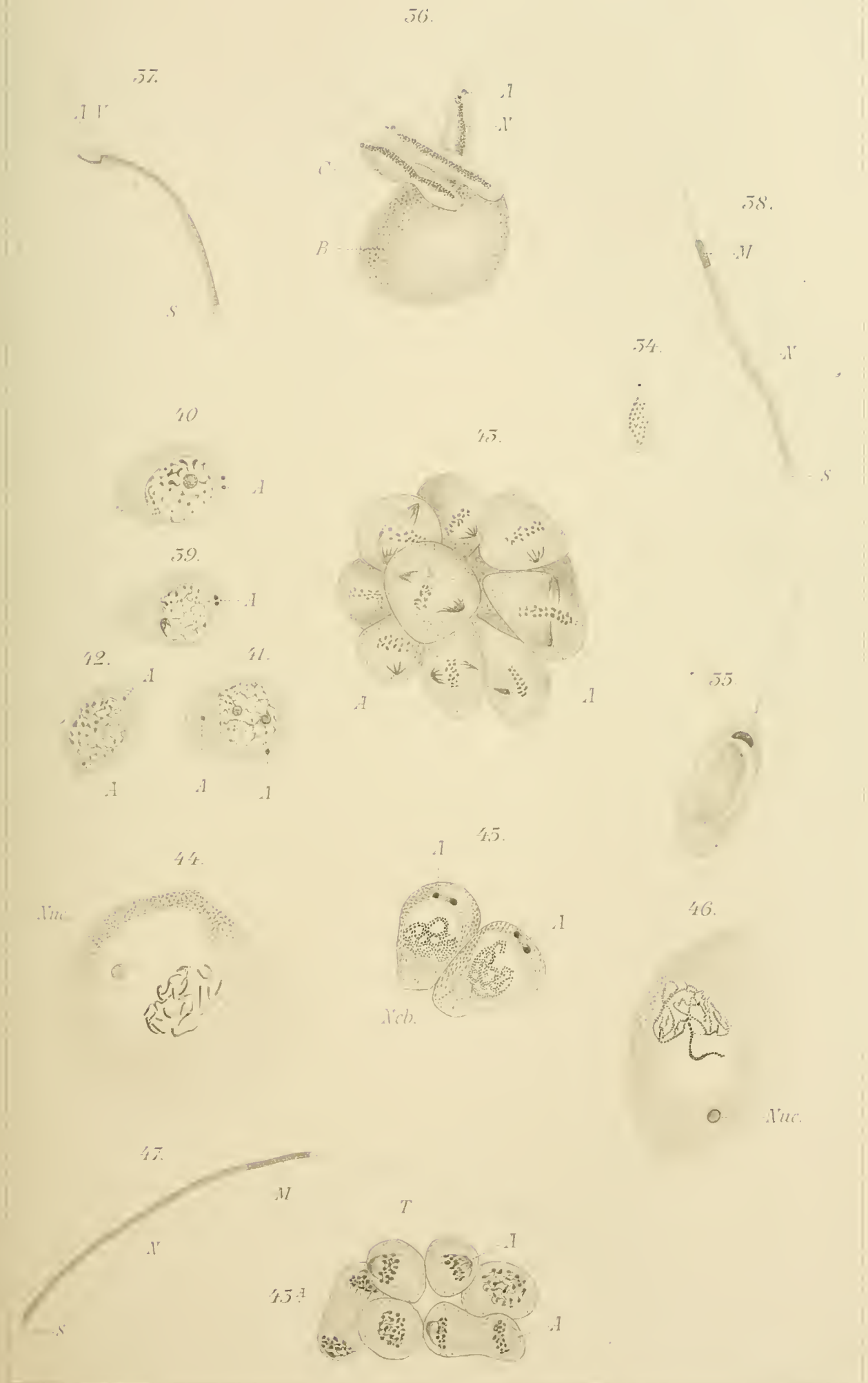
FIG. 43. Spermatosphere in the spermatocyte stage, showing archoplasm masses extending up the spindle fibres. The fibres come from the archoplasm.

FIG. 43A. Spermatid in the anaphase of karyokinesis, showing withdrawal of the spindle fibres into the archoplasm mass.

FIGS. 44 and 46. Artefacts, showing the protrusion of nucleoli.

FIG. 45. Spermatocytes of the first order, showing central spindle between the divided archoplasm masses. Nebenkern is also present.

FIG. 47. Late spermatozoön, showing tail, middle-piece, and head, also the spur (S) formed from the point of cytoplasm by which the spermatid had been attached to the blastophore.


56.
57.
A V
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Xnc.
46.
Nuc.
47.
M
N
S
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43A
A

THE OSTEOLOGY AND RELATIONS OF PROTOCERAS.

W. B. SCOTT.

(*Investigation aided by a grant from the Elizabeth Thompson Fund of the A.A.A.S.*)

No group of mammals offers greater difficulties in the way of arranging all its members in their order of relationship to one another than the Artiodactyla. These difficulties arise largely from the great number of extinct genera which are very imperfectly known from fragmentary remains, and as a consequence of this it is frequently impossible to determine the taxonomic values of resemblances and differences. The first step toward bringing order out of this chaos must consist in completing our knowledge of the structure of the many imperfectly known genera, and wherever it is possible, in carefully tracing out all the cases of parallel or converging development in one or other organ, where it can be shown to have taken place. In this way we may hope eventually to bring together such a body of facts as will clear up these complicated relationships. The Princeton Museum has recently obtained, thanks to the energy and skill of Mr. Hatcher, very extensive and wonderfully preserved collections of Tertiary mammals from the Western States, comprising many nearly complete skeletons of artiodactyls hitherto known only from scattered fragments. As soon as this material can be prepared, it is my purpose to render it available for comparison by careful description and full illustration. The perhaps tedious minuteness of such descriptions cannot well be avoided, if the object in view is to be effectively served.

The extraordinary genus *Protoceras* (Marsh) is one of those which will best repay exact and minute study, for, although its systematic position is far from clear, for reasons which will appear later, yet it throws much welcome light upon the mode of development among the artiodactyls, and shows how it has

happened that the different characters of structure are so variously and puzzlingly combined among the several groups of the order.

PROTOCERAS (MARSH).

The type specimen of the genus is an imperfect skull described by Marsh under the name *P. celer* (No. 5, p. 81), which is characterized by the presence of a small pair of protuberances, resembling horns, from the parietal bones. Subsequently Osborn and Wortman (No. 8) showed that the type specimen belonged to a female and described the male skull, which is one of the most curious fossils ever found and which displays very remarkable secondary sexual characters. They also described and figured the fore and hind feet, which differ in the most striking way from what the structure of the skull would lead us to expect. In the explorations of the Princeton Expedition of 1893 Mr. Hatcher collected a large number of female skulls and one nearly complete skeleton, the material which forms the subject of the present paper. During the season of 1894 Mr. Hatcher has added considerably to this material and has visited the spot where the type of the genus was collected, in order to establish its exact stratigraphical position, which was previously unknown.

I. *The Dentition.*

The dental formula is $i\,\frac{0}{3}$, $c\,\frac{1}{1}$, $p\,\frac{4}{4}$, $m\,\frac{3}{3}$, which differs from that believed to characterize *Gelocus* in an additional upper premolar, although it is not certain that $\underline{p\,1}$ may not have been present in the latter genus also.

A. *Upper Jaw* (Pl. XXI, Figs. 1 and 2). — The incisors have entirely disappeared, leaving no traces of alveoli in the premaxillaries. In the female the canine is rudimentary, but in the male it forms a long curved tusk, comparable to that of the tragulines, though rather shorter and of entirely different shape. While in the latter it forms a compressed scimetar-like blade, in *Protoceras* it is trihedral like that of the oreodonts, but relatively longer, more compressed and slender, and

is quite strongly everted laterally. As in the oreodonts, the canine is abraded upon the *posterior* face, which renders it probable that in the male the first lower premolar had taken on the form and function of a canine. The first premolar is much the smallest of the series and has a simple compressed and trenchant crown, implanted by two quite widely diverging fangs, and consisting only of the protocone without additions. The tooth stands by itself, being isolated by considerable diastemata both from the canine and from $\underline{p2}$. The second premolar is remarkably elongate in the antero-posterior direction, resembling in this respect the corresponding tooth of *Xiphodon* and in a less degree that of *Leptomeryx*. The crown is very low, compressed and trenchant; obscurely marked anterior and posterior basal cusps are separated by shallow grooves from the protocone. There is a strong internal cingulum running the whole length of the crown, thickened and elevated at the median point to form an incipient deuterocone, which is supported on a third fang. In some specimens the deuterocone can hardly be said to exist and the cingulum is feebly marked, and then the crown is carried on two fangs only. Seen from the outer side this tooth much resembles $\underline{p2}$ in *Leptomeryx*, except for its greater elongation, but in the latter the deuterocone is much larger and the cingulum absent. The third premolar is very much like the second, but with some modifications; thus the antero-posterior length is somewhat less, while the transverse width is somewhat greater, an increase which is chiefly due to the greater thickness of the protocone, though the deuterocone is also better developed. This tooth differs from $\underline{p3}$ in *Leptomeryx* in the much smaller deuterocone and in the presence of a complete internal cingulum. The fourth premolar is of the usual ruminant pattern, consisting of a single pair of crescents. A fairly developed cingulum is present in the inner crescent, which is very faint on the anterior side, but distinctly marked on the posterior. This cingulum is of course not homologous with that on $\underline{p2}$ and $\underline{p3}$. A comparison at once shows that the cingulum of $\underline{p3}$ is represented by the horns of the inner crescent on $\underline{p4}$ and that the inner cingulum of the latter is something superadded.

The upper premolars as a whole are very much like those of *Leptomeryx*, but more elongate and of somewhat simpler construction. In *Hypertragulus* they are still simpler and more trenchant, as they are in existing tragulines.

The molars are of the extreme brachyodont type, short antero-posteriorly and broad transversely, so as to present a nearly square outline; $\underline{m\,2}$ is slightly the largest of the series. The external buttresses (proto- and mesostyles) and the median ribs on the outer faces are very prominent and sharply defined; the internal pillar varies considerably in the different specimens, — in some being quite absent and in others strongly developed; the inner cingulum also varies much in strength and would appear to be more prominently marked in the males than in the females. The valleys are narrow and shallow and rapidly wear down to mere lines. The shape and proportions of these molars are closely like those of *Leptomeryx*, the principal difference between the two genera being that in *Protoceras* the inner crescents are rather more complete. *Dorcatherium* has molars of which the outer crescents are extremely like those of *Protoceras*, but the inner ones are thicker and more sloping. In *Hypertragulus* the molars are like those of *Leptomeryx*.

B. *Lower Jaw* (Pl. XXI, Figs. 1 and 4). — None of the specimens in the Princeton collection have the lower incisors or canines in position. Osborn and Wortman give the following description of them. "The inferior incisors present delicate spatulate crowns; the median [and] second incisors are slightly larger than the lateral incisor, which is very delicate. The canine has precisely the same delicate structure as the lateral incisor" (No. 8, p. 359). In the female $\overline{p\,1}$ resembles the corresponding tooth in the upper jaw, but is rather smaller and is implanted by a single fang. I have seen no example of this tooth in the male, but as has already been mentioned, the abrasion on the *posterior* face of the upper canine renders it probable that in that sex $\overline{p\,1}$ was caniniform. Even in the female it is considerably in advance of $\underline{p\,1}$ and nearly far enough forward to abrade the upper canine, being separated by a diastema from the lower canine and by a longer one from $\overline{p\,2}$. In *Gelocus* $\overline{p\,1}$ is very small and simple and stands close to $\overline{p\,2}$.

The second premolar is much more elongate antero-posteriorly than the first and is inserted by two widely separated fangs ; the crown is low and compressed, with trenchant edges, and is of very simple construction. The para- and metaconids are but obscurely developed, and a faint ridge on the inner side of the protoconid encloses an incipient valley. The third premolar, like $\underline{p3}$, is the longest (fore and aft) of the series, though the difference is less marked than in the upper jaw. In construction it resembles $\overline{p2}$, but all the elements are more distinctly differentiated and the posterior valley is deeper. The fourth premolar is shorter and wider than the third and has its component cusps more clearly demarcated ; the deuteroconid is well shown and the ridge which passes backward from that cusp extends to and fuses with the metaconid, so that the posterior valley forms a "lake."

The inferior premolars of *Leptomeryx* are in general very similar to those of *Protoceras*, but $\overline{p1}$, which stands isolated by diastemata, is much more reduced, and on the others the anterior and posterior basal cusps (para- and metaconids) are much more distinct and form sharp elevated points. In *Hypertragulus* $\overline{p1}$ is wanting, $\overline{p2}$ has a considerable diastema both in front of and behind it, and $\overline{p3}$ and $\overline{p4}$ are smaller and simpler than in either of the genera mentioned.

The molars are very low-crowned and have narrow, shallow valleys, which are worn nearly to the bottom at a comparatively early stage of attrition. The crowns are quite broad in proportion to their length, which increases regularly from $\overline{m1}$ to $\overline{m3}$. The internal crescents are thinner and less distinctly conical than in *Gelocus*. In the smaller and more abundant specimens the external pillar is but feebly developed, especially upon $\overline{m1}$, in the largest-sized jaws (perhaps old males) the pillar is well shown ; in such cases it is largest on $\overline{m3}$, smallest on $\overline{m1}$. The talon of $\overline{m3}$ is large and consists of two elements, which are more or less distinctly separated from each other. In *Leptomeryx* the lower molars differ from those described chiefly in their relatively less width and greater height, in the absence of the external pillar, and in the complete separation of the two elements which compose the talon of $\overline{m3}$.

C. The *Milk Dentition* (Pl. XXI, Fig. 5) of the lower jaw is not represented in the collection, and that of the upper jaw only partially so. The second milk molar is very elongate and compressed and consists of three elements, the protocone in the middle, with a basal cusp in front of and behind it, all of which have acute apices and trenchant edges; the internal cingulum is strongly developed and forms a prominent ridge. This tooth, aside from the cingulum, is strictly in the "triconodont stage," though, of course, no phylogenetic significance can be attributed to this fact. The third milk molar ($\underline{d3}$) is very peculiar and not quite like the corresponding tooth of any artiodactyl with which I have compared it, though most resembling that of *Gelocus*, *Leptomeryx*, and the tragulines, and thus is quite distinct from the type of $\underline{d3}$ in the existing Pecora. As in the former and in very many ancient genera, such as *Dichobune*, *Dichodon*, *Cænotherium*, *etc.*, the crown bears three external cusps, the protocone and tritocone and in front of the former an anterior basal cusp, for which I have proposed no name, because of the comparative rarity of its occurrence. The tritocone is shaped precisely like the external crescent of $\underline{p4}$, while the protocone is of compressed conical form. The postero-internal cusp, or tetartocone, is likewise crescentic, and thus the hinder half of the tooth is like the entire crown of $\underline{p4}$. So far, this description would apply equally well to *Leptomeryx*, the difference between the two genera consisting in the great prominence of the inner cingulum on the anterior half of the crown in *Protoceras*, which is thickened and elevated at two points to form two incipient cusps; of these the posterior, opposite the protocone, is of course the deuterocone, while the anterior one has not been named.

In the Pecora $\underline{d3}$ has the form of a permanent molar. I have elsewhere shown (No. 10, p. 370) that Rütimeyer has probably attributed too great taxonomic value to this fact, since in the same family both types of $\underline{d3}$ may occur, as, for example, in *Oreodon* and *Merychyus*. Yet, nevertheless, the feature is important and it is not without significance that while *Protoceras* resembles the Pecora in many details of skull structure, the dentition, and especially the milk teeth, are those of the more ancient genera.

The fourth milk molar is molariform and differs from the permanent ones only in size.

II. *The Skull* (Pl. XXI, Figs. 1–3).

No genus hitherto known from the White River formation has such a modernized type of skull as *Protoceras*, which, indeed, is in some respects in advance of the existing hornless deer, such as *Moschus* and *Hydropotes*, and in one or two points exceeds all the *Cervidæ*, approaching to the highest type of artiodactyl structure, the Cavicornia. At the same time, remnants of the primitive condition are by no means wanting, and the resulting combination is a very curious one. Another characteristic feature of the skull in *Protoceras* is the extreme degree to which the sexual differences are carried. Such an amount of sexual difference is altogether unparalleled among the ancient artiodactyls and is not attained even among the modern *Cervidæ*. The modernization of the skull, which has been referred to, consists in the following characters : (1) the shortening and rounding of the cranium ; (2) the backward shifting of the orbit, which is removed entirely behind the line of the molar teeth ; (3) the great elongation of the facial region, due not only to the shifting of the orbit, but also to the lengthening of the muzzle; (4) the bending downward of the face upon the cranio-facial axis, which does not occur in the *Cervidæ*.

The material at command displays three well-marked types of skull-structure, two of which are undoubtedly due to sexual distinctions, while the significance of the third is not yet entirely clear. It will be most convenient to begin the description with an account of the unmistakably female skulls, since these are the less extremely specialized type, and therefore best fitted to display the fundamental characters of structure. Of these females, there are five finely preserved skulls in the Princeton collection, with fragments of several others. The females are much more abundant than the males, as would be expected from the analogy of recent ruminants. Among these skulls there is a considerable degree of variation, and perhaps more than one species is represented by the

specimens, but, as this is not clearly shown, it will be better to treat the variations as individual.

Aside from certain peculiar specializations, the general character of the skull has already been pointed out in the list of modernizations given above. As a whole, the skull is very long and narrow, tapering rapidly toward the anterior end, where the muzzle becomes extremely slender. The face is constricted in front of $\underline{P4}$, and again in front of $\underline{P2}$, so that the base of the skull has a remarkably llama-like appearance. The upper contour rises at the forehead, the cranium being considerably elevated above the level of the face, though less than in the antlered deer. The occiput is of antique type, high, narrow and with its upper portion deeply concave and projecting backward, very different from the low, broad, and convex occiput of the tragulines. However, as in that group, the upper margin of the foramen magnum forms the hindermost part of the skull. The lambdoidal crest is prominent, the sagittal much longer and higher than in any existing selenodont, except the camels, and the temporal ridges are likewise unusually prominent.

The basioccipital is quite broad and massive, in some specimens faintly keeled, in others with a shallow groove along the median line; the very small tympanic bullæ do not encroach upon it at all. The body of the bone is relatively narrower and deeper in the vertical direction than in the antelope or deer skull, or even than in *Moschus*. The condyles are large, especially in the vertical dimension, and are quite widely separated below, though less so than in the deer. The articular surface is continued forward upon the two tubercles of the basioccipital, as in the deer and in some antelopes. On the outer side of each condyle there is a curious emargination of the articular surface, which invades the process along the line where the dorsal and ventral surfaces meet. This emargination occurs, but in a much less marked degree, in *Moschus*, *Cariacus* and other recent ruminants. The exoccipitals are low and narrow, as compared with their relatively great breadth in the recent Pecora; in the median line they are quite strongly convex, bulging out here to form a fossa for the

vermis of the cerebellum ; laterally they become concave, so as to enclose a deep fossa on each side. The paroccipital processes are long, slender and laterally compressed ; in shape and relative position, they are quite like those of *Moschus*. The inferior surface of the exoccipital, between the paroccipital and the condyle, is not wide, but is unusually long in the fore and aft direction ; this space is not bounded in front by a ridge running from the paroccipital to the basioccipital, as is the case in many deer. The foramen magnum is rather small and of subcircular shape ; its dorsal border is notched in the middle line and on each side of the notch is a more or less prominent and thickened process. The supraoccipital is high, but narrow and very thick, especially in the upper portion, where the diploëtic structure is well developed. The posterior surface is concave and small "wings" are formed on the sides. The parietals do not take part in the formation of these wings, because the supraoccipital is reflected over upon the top of the skull and forms an appreciable part of the cranial roof, not only in the middle line, as in *Moschus*, but also upon the sides, as in the tragulines. The summit of the lambdoidal crest is formed entirely by the supraoccipital. None of the existing Pecora has such a primitive type of occiput as *Protoceras*. In all of them the occiput is much lower and broader ; there are no such marked median convexity and deep lateral fossæ, and the crest is almost or entirely obliterated. Even the tragulines have this region shaped more like that of the Pecora than has *Protoceras*. On the other hand, the shape found in the latter occurs also in many ancient artiodactyls, such as the oreodonts, but in this family the occipital wings are much more prominent and the parietals take part in the formation of them. The Deep River genus, *Blastomeryx*, which is an undoubted member of the Pecora, has an occiput strangely like that of the oreodonts.

The cranium, which is remarkably well-rounded and capacious for a White River animal, though not very large, judged by the modern standard, is roofed in principally by the large parietals, which cover nearly the whole cerebral fossa and extend farther forward than in the existing Pecora, though the parietal

zone is somewhat shortened as compared with that of the tragulines, and very much so when contrasted with the long parietals of such genera as *Oreodon* or *Ancodus*. For most of their length the parietals unite to form a thick and prominent sagittal crest, which is cancellous internally and encloses a small sinus. Anteriorly, near the frontal suture, the crest bifurcates into two temporal ridges, which are recurved and overhanging, thus enclosing a deep grove in which a goose-quill may be concealed. These temporal ridges are confined to the parietals, and become thickened and more rugose at the points where in the male skull the large protuberances arise. These thickened areas differ somewhat in shape and in prominence in the various specimens, but in none of the skulls before me do they rise into protuberances at all comparable even with those of the specimen figured by Marsh (No. 6, Pl. XXI, Fig. 1). In most of the female skulls the parietals extend quite far down upon the sides of the cranium, though there is variation in this respect, and in front of the squamosals send down narrow processes to meet the alisphenoids. The latter are antelopine rather than cervine in shape, which is no doubt due to the great amount of backward displacement which the orbit has undergone. The ascending process of the alisphenoid is narrow, but its lower portion is reflected backward, and internally to the glenoid cavity extends almost to the tympanic. The pterygoid process is also narrow and has decidedly less vertical height than in recent Pecora.

The basisphenoid forms a rather slender and almost cylindrical rod, which is distinctly narrower than in the deer. The tympanic is much like that of *Moschus;* the exceedingly small bulla is but seldom well preserved, so that it is not surprising that Marsh should have concluded that "there were apparently no auditory bullæ" (No. 5, p. 82). The bulla is produced anteriorly into a long, slender spine, and on its postero-external side is a deep pit for the tympano-hyal. A long auditory meatus occupies the space between the postglenoid and post-tympanic processes of the squamosal, but does not form a complete tube, the upper part being covered in by the squamosal. Sir Victor Brooke's account of these structures in the musk deer

may be quoted here with advantage. "In *Moschus* the auditory bulla is remarkably small, the petrous portion of the periotic being visible, when viewing the base of the skull, for nearly the entire length of the bulla from before backwards. The tympanic is considerably prolonged to form the inferior floor of the external auditory meatus" (No. 1, pp. 522–3). In *Protoceras* the approximation of the tympanic and basioccipital is too close to allow the periotic to be seen, except in one specimen where the exposure is clearly due to a displacement of the surrounding parts. As in *Leptomeryx* and in the Pecora generally, the tympanic is hollow and not filled with cancellous tissue, but the bulla of *Leptomeryx*, though decidedly smaller than in *Tragulus*, is relatively much larger than in *Protoceras*. The mastoid portion of the periotic forms a narrow and more or less rugose strip between the exoccipital and the squamosal, and is continued upon the anterior face of the paroccipital process for some distance below the posttympanic.

The squamosal forms a varying part of the side wall of the cranium, the ascending plate rising higher in some specimens than in others. The parietal suture is regularly arched upward and backward. As in the deer, the bone forms a truncated process at the anterior end of this suture, where the cranial wall turns inward to form the front boundary of the cerebral fossa; apparently, however, the alisphenoid takes less share in the formation of this process than is the case in *Cervus*. The root of the zygomatic process is thicker and less concave on its upper side than in the recent Pecora and the process itself is heavier than in those animals, in which it is remarkably weak; as to length, it is longer than in the cavicorns and shorter than in the deer, the orbit occupying a position intermediate between the rather anterior place it holds in the latter, especially in the hornless forms, and its very posterior position in the former. The glenoid cavity is typically ruminant in character; in front it is broad and slightly convex, passing behind into a concavity. The postglenoid process is longer and considerably heavier than in most recent ruminants, though very much less so than in such types as the oreodonts or *Ancodus*. The process does not, as in the deer, bound the

whole width of the glenoid cavity, nor, as in the cavicorns, is it confined to the postero-internal angle, but is placed somewhat internal to the median line. The posttympanic process is very short and slender and is closely applied to the mastoid portion of the periotic. In all the recent Pecora there is a large opening between the auditory meatus and the inferior margin of the squamosal for the passage of nerves and blood-vessels ; in *Leptomeryx*, *Hypertragulus*, and the recent tragulines no trace of this opening is to be found. *Protoceras* agrees with the latter in this respect, the auditory meatus being in contact with the squamosal in front, behind, and to some extent, above.

The jugal is heavier than is usual in existing ruminants ; its posterior branch extends backward beneath the zygomatic process to the outer edge of the glenoid cavity. Beneath the orbit the jugal projects outward into quite a broad horizontal shelf, corresponding to the prominence of the supraorbital border. The postorbital process is short, and deeply notched to receive the anterior end of the zygomatic process. The orbit, which is completely encircled by bone, is bounded behind more by the postorbital process of the frontal than by that of the jugal. The vertical portion of the jugal, which articulates with the maxillary, is rather small and is not much expanded upon the face ; this is due to the rather low position held by the orbit, which is not so much elevated as in the typical deer. This has an effect also upon the conformation of the posterior nares. The lachrymal, which is quite large, is not depressed to form a pit or fossa, as it is in so many of the recent deer and antelopes, as well as in the oreodonts. It has a limited suture with the nasal, instead of being separated from it by a vacuity, such as occurs in *Leptomeryx*, *Hypertragulus*, and most of the existing Pecora. Short as are the nasals, they are long enough to show indications of such a fontanelle, had it existed. No great taxonomic value can be attached to this character, as within the limits of the same family we may find some genera with and others without it, as, for example, in the oreodonts, *Merychyus* and *Leptauchenia*, with its allies, have the vacuity, the other genera are without it.

The frontals are large, though less extended antero-posteriorly than in the modern Pecora and forming less of the roof of the cerebral fossa; they reach from a little behind the orbits to considerably in front of them, but the temporal ridges do not encroach upon them at all. They are transversely concave and at their outer edges are raised and thickened to form the rugose upper borders of the very prominent orbits. Though far less so than in the males, these projections are much more striking than in the recent Pecora. In the median line of the frontals not far from the parietal suture is a small, rounded and more or less rugose eminence, which varies in size in the different individuals, though it is not prominent in any of the females which I have seen. Judging from Marsh's figures and description, it would appear to be better developed in the type specimen. As in the oreodonts and primitive artiodactyls generally, the supraorbital foramina are placed near to the median line and the vascular grooves which run forward from them are very distinctly marked. There is a small frontal sinus.

The nasals are very remarkable; they are broad from side to side, but exceedingly short. The median portion is elevated, transversely convex, and projects slightly beyond the maxillary suture, the free portion rapidly tapering to a point. The lateral portion is more depressed and flattened and unites suturally with the maxillary, frontal and lachrymal. Owing to their extreme shortness, they are, of course, very far removed from any contact with the premaxillaries. Though the reduction of the nasals is less extreme than in the saiga antelope, this part of the skull has a striking resemblance to that animal, and as in it, the nasal chamber has no osseous covering for nearly the whole of its length. It can hardly be doubted that *Protoceras* must have possessed a proboscidiform muzzle much like that of the saiga, and as in that genus, the turbinal bones were doubtless greatly shortened. In the moose (*Alces*) the nasals and turbinals are considerably reduced in length, though to a much less extreme degree.

The edentulous premaxillaries are very much like those of the modern ruminants, except that they are of unusually small

size; they project but little in advance of the canines and so do not add much to the length of the muzzle. The alveolar portion forms a thin, depressed, and delicate plate, with regularly rounded free margin, which in shape resembles that of *Cariacus*. The spines, on the other hand, are relatively quite broad and very thin, and hence the incisive foramina form narrow crescentic slits, the outer wall of which is formed for nearly half their length by the maxillaries; these foramina are thus much less widely opened than in most ruminants, which is due to the narrowness of the spines in the latter. The ascending ramus of the premaxillary is short and slender and forms an obtuse angle with the alveolar portion; it is separated by nearly half the length of the skull from the shortened nasal. In the saiga the premaxillaries are much heavier and wider proportionately than in *Protoceras* and the ascending portion makes a still more open angle with the alveolar portion; in *Hydropotes* the ascending ramus is wider.

The maxillaries have considerable resemblance, when seen in profile, to those of the saiga antelope, but owing to the extremely brachyodont dentition, the alveolar portion is much lower, and as the orbit is considerably less elevated above the level of the palate, the descent of the upper or free margin of the maxillaries toward the front is much more gradual. Another difference between the two genera consists in the more complete reduction of the nasals in the modern type and the larger size of the lachrymals, owing to which the latter form the upper margin of the nasal chamber for some distance. In *Protoceras* the nasals articulate with the maxillaries and thus cut off the lachrymals from the border of the nasal chamber. The vertical plate of the maxillary above the sinus or antrum is very thin and delicate, but has a round, seam-like free border, which is a little thicker than the rest of the plate. As Osborn and Wortman conjectured, there are no such great and massive protuberances on the maxillaries as occur in the male, but they are faintly indicated by a slight upward arching of the border; posterior to this the margin is notched and from the notch descends a shallow groove, doubtless marking the course of a blood-vessel. The masseteric ridge is very prominent, though

less so than in the male, and extends far forward, nearly to $\underline{p3}$, terminating in a short spine, which again is very much less developed than in the male. Above the masseteric ridge the ascending plate of the maxillary is sharply inflected to form a horizontal surface and toward the median line again assumes a vertical direction, thus giving to the bone a step-like section and greatly narrowing the dorsal part of the nasal chamber. The horizontal surface thus formed ends anteriorly in a deep fossa, to which Marsh has already called attention, but in his specimen the fossa appears to be somewhat differently shaped and more distinctly demarcated. In advance of $\underline{p2}$ the maxillary is very low, its upper border curving gently downward to the premaxillary suture, and its outer surface is flat, there being no large canine alveolus to cause a swelling. This region of the jaw is very long and it bears the chief share in the characteristic elongation of the muzzle. In one of the specimens, which may possibly represent a distinct species, the portion of the maxillary which is in advance of $\underline{p2}$ is shorter than in the usual type of female skull, and the arching of the upper border which represents the great maxillary protuberance of the male, is thicker and has a more rugose margin. From this process the descent of the border to the premaxillary suture is more abrupt. The lower border of the maxillary fossa, which is a continuation forward of the masseteric ridge, though not rugose like the latter, is more prominent and extends farther upward upon the maxillary process. In these respects this skull approximates the characters of the male more closely than do the long-muzzled females which are more commonly found. The teeth of this specimen are remarkable for the relatively feeble development of the internal cingulum upon the upper premolars and its unusual prominence on the molars.

The palatine processes of the maxillaries form nearly the whole of the bony palate, the premaxillaries and palatines taking but little share in it. The palate is widest between the first molars of the opposite sides and gradually narrows in each direction from that point, while in front of $\underline{p2}$ it is constricted and becomes very narrow; it is unusually flat both transversely and antero-posteriorly. The faintly marked rugose ridges

which indicate the limits of the soft palate in front of the premolars, are in many existing ruminants placed quite close to the median line, so that part of the ventral surface of the maxillary is not covered by the soft palate. In *Protoceras*, on the other hand, these ridges run along the angle formed by the meeting of the horizontal and vertical surfaces of the bones, which thus do not curve into each other so gently as in the modern forms mentioned. The posterior palatine foramina occupy a very unusual position. Ordinarily in ruminants, as in other mammals, they are situated in or near the maxillo-palatine suture, but in *Protoceras* they are placed just internal to $\underline{p^1}$ and from them deeply marked vascular grooves run forward to the incisive foramina. These, perhaps, represent the foramina which in *Oreodon* and *Ancodus* occur opposite $\underline{p^3}$.

The palatines are rather small bones and hardly do more than form a border around the posterior nares; they are separated from the molars by a considerable strip of the maxillaries, whereas in the Pecora the palatines are almost in contact with the molar fangs. The posterior nares are much more ruminant than suilline in character and yet are peculiar in many ways. The canal is very elongate antero-posteriorly, but is very narrow and of considerably less vertical height than is usual in the Pecora, a feature which is correlated with the relatively small elevation of the cranium above the level of the face. The anterior border of the opening is placed between the second pair of molars and has a short thickened median spine. The palatine notches are narrow and shallow, as compared with those of the deer; they are not formed, as in the Pecora generally, by a constriction of the palatines, but lie between the latter and the maxillaries. The pterygoids are thin, slender plates of bone of no great height, which terminate in slightly thickened and everted hamular processes. There are no pterygoid fossæ and no such separation between the distal ends of the pterygoids and alisphenoids as occurs in the Tylopoda.

The mandible is altogether pecoran in character, resembling that of *Leptomeryx* but little and that of the oreodonts not at all. The horizonal ramus is very long and quite slender, in accordance with the great proportional length of the molar-

premolar series and their very brachyodont condition. In front of $\overline{p2}$ the thin edentulous upper border descends quite abruptly, rising again slightly to form the alveolus of $\overline{p1}$. The incisive alveolar border is slightly widened and somewhat spatulate in form, though retaining a greater vertical depth than is usual in the Pecora; the widening is very limited, as would be inferred from the extreme slenderness of the upper jaw in this region. The symphysis is quite long and rather oblique, and in some aged individuals the two rami of the jaw appear to be coössified. Two rather large mental foramina occur in the symphyseal region, the first underneath and the second slightly behind $\overline{p1}$. The horizontal ramus has the undulating lower margin and anterior taper found in existing ruminants, but is rather stouter than is common in that group and is quite unlike the straight, slender ramus of *Leptomeryx*. The ascending ramus is rather wider than in the most of the Pecora and does not rise so high as in the cavicorns, which is due to the fact that the glenoid cavity and base of the cranium are not so much elevated above the level of the molars as in those forms. The angle is thin and entirely inconspicuous, not projecting behind the line of the condyle, being shaped very much as in *Moschus*, which is a striking difference from *Leptomeryx*. In the latter the angle projects very far back of the condyle, in a way which recalls that of the ancestral Tylopoda. The masseteric fossa is less profoundly marked than in *Leptomeryx* and extends farther downward and forward; it is somewhat more distinctly imprinted upon the jaw than in the musk-deer, but has much the same shape and extension. The coronoid is very low and tapers abruptly to a blunt point. This is the only character in which the mandible of *Protoceras* resembles that of the oreodonts; the sigmoid notch is much narrower than in *Leptomeryx*. The condyle is like that of the Pecora, but in correlation with the better development of the postglenoid process, the articular surface is more reflected upon the posterior face of the condyle. While in the Pecora the latter is slightly concave transversely, convex antero-posteriorly and thus saddle-shaped, in *Protoceras*, on the other hand, it is plane transversely and more strongly convex in the fore and aft direction.

The *foramina* of the skull are antelopine rather than cervine in character, which is doubtless due to the backward shifting of the orbit, this displacement having proceeded farther than in the deer, especially the hornless genera. The infraorbital foramen is single and occupies rather a posterior position; it lies above the interval between $\underline{p3}$ and $\underline{p4}$ (in the deer it is above or in advance of $\underline{p2}$) and is roofed by the tubercle in which the masseteric ridge terminates. The optic foramen is lower than in most antelopes, in correspondence with the less elevated position of the orbit. The foramen lacerum anterius is rather small and rounded, while in the deer it is very large and of irregular shape, especially so in *Moschus*, where it forms a great fissure. As in the Pecora generally (and indeed most artiodactyls) the foramen rotundum is not present, though its position is in some specimens marked by a pair of minute openings. The foramen ovale is quite large and placed internally to the glenoid cavity; it varies in shape, being in some individuals nearly round and in others narrow and elongate. In spite of the very small size of the auditory bullæ, the foramen lacerum medium and posterius are mere slits, much narrower than in *Cariacus*, and the bulla is not channelled by the carotid canal. A postglenoid foramen is present, though of small size. The condylar foramen occupies a position where it is concealed by the prominence of the inferior portion of the condyle. The squamosal is perforated by two large vascular openings above the root of the zygomatic process. The remarkable position of the posterior palatine foramina has already been described; it only remains to mention an irregularity in the two sides which sometimes occurs, — thus, in a male skull the left foramen is fully a quarter of an inch behind the right.

The second type of skull is that of the male, the knowledge of which we owe to Osborn and Wortman. Their beautifully preserved specimen is still by far the best that has been discovered, and is one of the most remarkable and curious of mammalian fossils. The striking resemblance of this skull to a miniature *Uintatherium* has been commented on by the authors mentioned, and is an excellent example of a superficial parallelism between two forms which are as widely separated

as two ungulates well can be, since they have no common ancestor later than the most ancient Condylarthra.

The most obvious difference in skull structure between the two sexes lies in the great development of osseous protuberances from various parts of the head in the male, all of which are, however, faintly indicated in the female. The latter is far less bizarre in appearance, and aside from the shortened nasals (a character which is found still more strongly marked in the saiga antelope and to a less degree in *Alces*), does not depart in any radical way from the modern hornless Pecora. Indeed, most of its deviations from the skull-structure of those animals is in the direction of the higher members of the same group. Besides the protuberances, the various ridges and processes for muscular attachments are naturally much stronger in the male, as is to be seen in the greater prominence, thickness, and rugosity of the lambdoidal, sagittal, temporal, and masseteric crests. From the temporal crests of the parietals arise a pair of horn-like protuberances, which in the female are mere roughenings of the crests. These processes are laterally compressed, of elongate oval section, and with their long axes running in the direction of the temporal ridges and oblique to the sagittal suture; their free ends are rounded and roughened. The second pair of protuberances are formed by the great development of the supraorbital borders of the frontals, which are drawn out into a pair of large, depressed, wing-like bodies, completely overhanging the orbits. These processes are relatively better developed in the female than any others of the paired protuberances. The posterior median elevation of the frontals is not much more conspicuous in the males than in some females. A third pair of conical, horn-shaped processes arise from the antero-external angle of the frontals, where they unite with the nasals and lachrymals. Feebly-marked indications of these processes are to be seen in the female, more decidedly in some individuals than in others. A fourth pair of protuberances are formed by the short, thick, conical processes which are given off from the anterior ends of the masseteric ridges of the maxillaries. These are likewise to be found in the female, though very much less strongly

developed. From the upper margin of the maxillaries arise the most anterior and the largest of the protuberances. "The whole conformation of the maxillaries is, so far as we know, unique among the mammalia; the superior borders curve sharply upward into two powerful plates of bone, concave on the outer side, convex on the inner, and rising to the level of the parietal processes, with a concave posterior and convex anterior border" (Osborn and Wortman, No. 8, p. 357). The concavity of the outer surface is interrupted by two convex ridges, one of which is the continuation upward and forward of the masseteric ridge, though its smooth surface shows that it is not a part of that ridge, and the other is the bulging caused by the alveolus of the large canine. These maxillary protuberances are, as we have already seen, shown in the female skull by a slight upward arching of the borders of the maxillaries; in some specimens they are quite distinct, and have slightly thickened and rugose margins, though not in any degree approximating their great size in the male. In *Uintatherium* the maxillary protuberances are rounded and horn-like, while in *Protoceras* they are plates. *Sus larvatus*, a recent species from Africa, has maxillary protuberances which are thick and very rugose. The infraorbital foramen is slightly more anterior in position in the male, and in that sex the premaxillary spines are rather narrower, so that the incisive foramina are somewhat more widely open. All the bones of the skull are thicker and more massive, as a result of which the male skulls are seldom so distorted by pressure as the female specimens very generally are.

Still a third kind of skull-structure is presented by the type specimen of *Protoceras celer*, as described and figured by Marsh (Nos. 5, 6). This specimen is imperfect and some points of importance cannot be determined from it. On the whole, it agrees best with the females which have already been described, but in none of the latter which I have seen are the parietal protuberances anything more than mere roughenings of the temporal crests, while in the type specimen they are fairly well developed, conical eminences, which are described as "a pair of small horn-cores, situated not on the frontals,

but on the parietals, immediately behind the frontal suture. . . . The horn-cores are well separated from each other, and point upward, outward and backward, overhanging somewhat the temporal fossæ. They are conical in form, with obtuse summits" (No. 5, p. 81). Osborn and Wortman, who have compared the type with their own specimens, report that these parietal "horn-cores" are about equal in size to the anterior frontal protuberances of the male skull figured by them, and are therefore very much smaller than the parietal protuberances of that animal and of quite a different shape. In Marsh's specimen, so far as can be judged from the figure (No. 6, Pl. XXII, Fig. 1), the median frontal eminence would appear to be more prominent than in the females which have been described above and the maxillary fossa is of a different shape and more distinctly bounded behind. The thinness of the maxillaries precludes the supposition that there can have been any great protuberances upon those bones.

Osborn and Wortman conclude that the type specimen was the skull of a female, and this result is altogether probable. At the same time, however, it is remarkable that among the numerous undoubted female skulls which have been collected, not one should show the conical, horn-like protuberances on the parietals which characterize the type. More extensive material will be required to determine the significance of this feature. It may be only an individual variation, in which the female has approximated the characters of the other sex, as we have already seen to occur sometimes in the case of the maxillary protuberances. Or, in the second place, the type specimen may represent the female of a species distinct from that to which the individuals described in this paper belong. I was at one time tempted to believe that the type might be the male of a more ancient genus than the specimens which Osborn and Wortman referred to *Protoceras*, and that it would be found to have come from the *Oreodon* beds. Mr. Hatcher has, however, lately visited the spot whence the type was taken, and writes me that it is in the *Protoceras* beds, well up toward the top.

MEASUREMENTS.

	MALE.	FEMALE No. 1.	FEMALE No. 2.	MOSCHUS.
Length of skull from occipital condyles	0.215	0.223	0.225	0.137
Breadth of skull at supraorbital margins	.111	?.072	.080	.052
Length of face from anterior border of orbit	.130	.146	.152	.075
Length of cranium from occipital crest to front margin of orbit	.100	.080	.075	.069
Occiput, height		.055	.055	.034
Occiput, breadth at base		.048	.046	.036
Sagittal crest, length	.030	.030	.033	
Frontals, length in median line	.050			.042
Nasals, length in median line	.030			.055
Palate, breadth at $\underline{m1}$	.028		.034	.028
Maxillary, length on alveolar border	.120	.131	.125	.067
Premaxillary, length in front of canine	.018	.020	.018	.022
Diastema between canine and $\underline{p1}$, length	.012		.015	
Diastema between $\underline{p1}$ and $\underline{p2}$	.018	.018	.018	
Mandible, length		.195		.118
Mandible, height of condyle above lower border		.056		.036
Mandible, breadth of angle		.052		.027
Mandible, depth below $\overline{m3}$		.026		.015
Mandible, depth below $\overline{p2}$		.022		.012
Diastema between $\overline{p1}$ and $\overline{p2}$, length		.027		

N.B. — The measurements of the male are taken in part from Osborn and Wortman.

III. *The Brain.*

No specimen of the brain-cast is preserved in the Princeton collection. Osborn and Wortman's figure shows that it was decidedly more advanced than in the case of any other White River genus so far known. Their account is as follows: "The brain is deeply convoluted. We observe upon each hemisphere four longitudinal gyri; these, according to Owen's nomenclature, would be the median, medilateral, suprasylvian, and sylvian" (No. 8, p. 355). A remarkably modern feature of this brain is in the width of the cerebral hemispheres, as compared with their length, and in this respect, as well as in its richness of convolution, it is superior to the brains of the small modern deer, such as *Moschus, Hydropotes, Cervus humilis, etc.*

The sulci give more precise means of comparison than do the gyri. Krueg describes the fissures of the traguline brain as follows: "Die Figur zeigt, dass auf der oberen Seite nicht nur die dahin gehörigen Hauptfurchen, sondern auch ein Theil der auf der Medianseite gelegenen Fissura splenialis sichtbar wird. Die Fissura coronalis ist bei allen Zeichnungen mit dem oberen Fortsatz der Fissura suprasylvia verbunden. . . . Die Fissura suprasylvia zeigt keine Marke zwischen Körper und hinterem Fortsatz. Ausser der sehr kurzen Fissura lateralis findet sich nach hinten und aussen von ihr noch eine accessorische Furche. . . . Die Hauptfurchen an der Lateralseite scheinen alle vorhanden zu sein. . . . Der Gesammthabitus der Furchen sowohl als der äusseren Umrisse *ist ausserordentlich ähnlich dem der Elaphier*" [*i.e.*, Cervidæ.] In the smaller deer, such as *Moschus*, *Elaphodus*, *Cervus humilis*, the splenial fissure (sulcus calloso-marginalis) extends over upon the dorsal surface of the hemisphere, as in the tragulines, the condition which Krueg calls "supination," while in the larger members of the group it is confined to the median side ("pronation"). "Was die übrigen Furchen anlangt, so ist der Processus acuminis fissuræ Sylvii gewöhnlich lang ausgezogen; so dass er fast die Kuppel der Fissura suprasylvia erreicht; es pflegt keine accessorische Furche zwischen beide eingeschoben zu sein. Die Fissura diagonalis ist mit ihrem Hinterende durch einen nach oben gerichteten Fortsatz der Fissura suprasylvia verbunden" (No. 4, pp. 315–317).

Osborn and Wortman's figure shows that the principal sulci in *Protoceras* correspond closely with those of the larger deer. There is no "supination" and the splenial fissure does not appear on the dorsal surface; the lateral fissure is long and a well marked accessory furrow lies between this and the suprasylvian. The latter is connected anteriorly with the coronal sulcus. The diagonal sulcus is plainly visible in the view from above, but does not quite reach the suprasylvian, and the acuminate process of the sylvian fissure is long. The convolutions are thus distinctly of an advanced and modern type. The cerebellum is not known, but as Marsh has suggested, its small size is indicated by the narrowness of the occiput.

IV. *The Vertebral Column.*

The skeleton which forms the principal subject of this paper retains twenty-one of the presacral vertebræ. We may conjecture with considerable confidence that the entire number of such vertebræ was twenty-six, and of the five missing ones several are represented in other specimens. The neck is relatively longer than in the tragulines or *Leptomeryx*, shorter than in *Moschus*, and is rather slender. As its actual length is almost the same as in the musk-deer, its shortness as compared with the length of the skull is very marked. In general characters the atlas (Pl. XXI, Fig. 6) is like that of the Pecora. It is long in proportion to its breadth, which is due to the comparatively small lateral extension of the transverse processes. The anterior cotyles for the occipital condyles are broad, of considerable height, and widely separated both above and below. Their external borders are quite deeply notched, corresponding to the similar emarginations on the outer sides of the occipital condyles, to which attention has already been called. The ventral incision between the anterior cotyles is more deeply cut than in *Cervus*, but the articular surfaces for the accessory facets developed on the tubercles of the basi-occipital in front of the condyles are not reflected so far over upon the inferior arch of the atlas as in that genus. Owing to the depth of this anterior incision and the position of the surfaces for the axis, the inferior arch is not so elongate fore and aft as in the deer, nor is it keeled ventrally, and the hypapophysis is hardly more than a rudiment. The arch is strongly convex transversely and has two shallow fossæ upon each of its sides. The neural arch is likewise short from before backward; for though the incision between the anterior cotyles is not so deep as on the ventral side, that between the posterior pair is much deeper. Like the inferior arch, the neural is strongly convex from side to side and the two together form a nearly circular ring. The neural spine is remarkably well developed and conspicuous; it is much compressed, but quite high, and occupies about one half the length of the arch. On each side is an elevated ridge, enclosing a lyrate area, in the middle

of which the spine rises. As usual in the Pecora, the arch is perforated by foramina for the first pair of spinal nerves. The posterior cotyles for the centrum of the axis are more oblique and less directly transverse than in the Pecora; they are quite high, but of no great width, and the articular surface is reflected within the ring of the atlas, where it forms a broad, continuous facet for the odontoid process. There is, however, no facet upon the hinder face of the inferior arch for the centrum of the axis below the odontoid, such as occurs in the Pecora. The transverse processes have no great width, but they extend out more widely than in *Cervus* or in *Moschus*, especially toward the posterior end; they are pierced by foramina for the inferior branches of the first pair of spinal nerves, but there is no vertebrarterial canal, as there is in *Agriochœrus*, the swine, *etc.*

The axis (Pl. XXI, Fig. 7) is peculiar in many ways. The centrum is very long, much more so than in any other vertebra of the neck, and is broad and much depressed in front, contracted in the middle, and subcylindrical behind. The posterior surface is obliquely placed and deeply concave, forming a hemispherical pit for the centrum of the succeeding vertebra. The ventral surface of the entire centrum is marked by a prominent but very thin hypapophysial keel. The articular surfaces for the atlas are narrow and high, though extending less upward along the sides of the neural canal than in the Pecora. In the latter the two surfaces are fused together beneath the odontoid process, forming an uninterrupted facet, and their dorsal ends pass into the pedicels of the neural arch. In *Protoceras* the articular surfaces do not extend beneath the odontoid, but are completely separated by a wide and deep emargination, and a still more extensive notch divides the dorsal end of each surface from the neural arch. The odontoid process is neither conical nor spout-shaped, but rather semicylindrical, with bluntly rounded point. The upper surface bears a median ridge with a shallow fossa on each side of it, and the ventral surface is gently convex from side to side. This shape of odontoid is the half-way stage in the conversion of the conical into the spout-like form.

It is remarkable how many of the approximately contemporaneous Oligocene genera have reached the same stage in the transformation of the odontoid, and as I have elsewhere shown, this transformation is one of the most convincing cases of parallelism, it having been accomplished many times independently and always in the same way. *Protoceras* is in the same stage of the change as *Oreodon*, *Gelocus* among the Pecora, *Pœbrotherium* among the camels and llamas and *Mesohippus* among the horses. The resemblance is particularly close in the cases of *Gelocus* and *Mesohippus*, while in *Pœbrotherium* the process is wider and shorter, and in *Oreodon* it is broader and more flattened than in any of the genera named. The significance of this oft repeated change in the character of the odontoid process is to be sought for in the relations between the axis of the skull and the line of the neck. In the short-necked forms with conical odontoid, it will be found that the cranio-facial axis is continuous with the line of the neck, or, at most, that they form a very open angle, while in the long-necked forms with spout-shaped odontoid, the two lines meet at a more or less acute angle. It is, of course, of the highest importance to give the spinal cord a channel without sharp bends, and this is accomplished in long-necked ungulates by making the odontoid concave.

In *Protoceras* the neural spine of the axis is different, not only from that of the Pecora, but also from that of most ungulates in general, in forming a great hatchet-shaped plate; its upper margin descends in a gentle, regular curve, from behind downward and forward, describing an arc of about 45°. The free border is but slightly thickened and rugose and the whole plate is very thin and delicate, though in some cases the hinder border is much thickened. The posterior zygapophyses are prominent, present obliquely downward and outward and have facets which are slightly concave transversely. The transverse processes are short and very slender, and their bases have no great antero-posterior extension along the sides of the centrum. The foramina of the axis are quite different from those found in the Pecora. In the latter the pedicels of the neural arch are perforated for the exit of the second pair of spinal nerves,

and, as the vertebral artery "usually enters the neural canal between the arches of the second and third vertebræ," the transverse process is generally not perforated by the vertebrarterial canal, but is pierced anteriorly by the inferior branch of the second spinal nerve. In *Protoceras*, on the other hand, the pedicel is imperforate, the nerve passing out through the deep notch which separates the atlanteal facet from the neural arch. As the transverse process arises behind this point, it does not traverse the path of the inferior branch of the nerve, and hence there is no foramen for that branch. The process is perforated, however, by the vertebrarterial canal, both openings of which are present, and the artery probably entered the neural canal between the arches of the atlas and axis.

None of the specimens contain representatives of the third or fourth vertebra.

The fifth cervical is relatively short, both actually and proportionately shorter than in *Moschus*, an animal which agrees well with this species in stature. The centrum is of depressed cylindrical shape, is strongly opisthocœlous and the faces are markedly oblique to the long axis of the vertebra. On the ventral surface is a prominent, but very thin and fragile keel, which terminates posteriorly in a hypapophysial tubercle. The neural arch is broad and nearly plane on the dorsal side and carries a spine which is much higher than in *Moschus* and which tapers and rapidly becomes very slender, though its base occupies nearly the whole length of the neural arch. The zygapophyses are large and widely separated on the two sides; the anterior pair are the narrower and present obliquely inward as well as upward. As the obliquity of the centrum is more decided than that of the neural arch, the pedicels of the arch are very low in front, and hence the ventral sides of the prezygapophyses are separated from the centrum only by narrow notches. The postzygapophyses are larger and more prominent than the anterior pair and project directly downward, almost without obliquity. The neural canal is broad and low, especially in front; posteriorly the increased height of the pedicels and obliquity of the centrum give it greater height. The transverse process is so mutilated on both sides that its

exact shape cannot be determined. Enough remains, however, to show that its origin on the centrum is less extended in the fore and aft direction than in *Moschus* and in consequence the posterior orifice of the vertebrarterial canal occupies a more advanced position than in that genus.

The sixth cervical has a centrum of nearly the same length as the fifth and is of a similar depressed cylindrical shape. The anterior face is strongly convex and hemispherical in shape, with a large shallow pit, or sulcus, for the attachment of the intervertebral cartilage ; the posterior face is as strongly concave, but both faces are much less oblique with reference to the long axis of the centrum than on the fifth vertebra. The ventral keel is very feebly developed and does not terminate behind in a tubercle, but simply dies away. The neural arch is very short antero-posteriorly ; this is due to the manner in which the arch is cut away between the prezygapophyses. The axis of the neck undergoes a marked change in direction between the fifth and sixth vertebræ and this is provided for by the projection of the anterior zygapophyses on the latter. These are broader, slightly more elevated and less oblique than on the fifth, while the postzygapophyses are very much the same on both, except that on No. 6 the articular surface is continued down upon the neural arch, forming an accessory facet ; the pedicels of the neural arch are higher and hence the neural canal is more rounded and less depressed. The spine does not appear to be much higher, but is decidedly thicker and heavier and inclines forward to about the same degree. As is always the case on the sixth vertebra, the transverse process is very clearly divided into diapophysial and pleurapophysial elements. The former is a stout, depressed rod, which stands out nearly at right angles with the centrum and having the distal end expanded in the fore and aft direction ; this process is decidedly more prominent than in *Moschus*. The pleurapophysis is a very large plate, slightly convex on the inner side and concave on the outer, with thickened free border, especially at the angles. This element is developed very much as in the musk-deer, but is of greater vertical height and the inferior face of the centrum is more clearly demarcated from the origin of the plates.

The seventh cervical is the shortest of the series and has a centrum of a shape differing from that of all the others; it is narrow in front and the anterior portion is so sharply constricted that it appears to stand upon a short neck; behind this it immediately broadens and the posterior face is wide and depressed and displays facets for the heads of the first pair of ribs. The faces are still somewhat oblique in position. The neural arch is very short, being cut away between the prezygapophyses, as is also the case in the sixth vertebra, another marked change in the direction taken by the spinal column occurring at this point. The prezygapophyses are very large and widely separated; for most of their extent they face upward, with but little obliquity, but the articular surface is reflected downward upon the inner side of the process and presents inward. This accessory facet articulates with the corresponding one on the outer side of the neural arch of the sixth vertebra, which has already been described. The postzygapophyses are very small, hardly a third as large as the anterior pair. The neural spine is no higher than that of the sixth, but is much larger in every other dimension and looks like a truncated thoracic spine, which stands erect, instead of pointing forward as in the other cervicals. The transverse process is quite a long, depressed bar, which, as usual, is imperforate. This vertebra differs from the corresponding one of *Moschus* in the heavier neural spine, the much more decided lateral projection of its zygapophyses and the longer transverse processes.

The *Thoracic Vertebræ* (Pl. XXI, Fig. 8) were probably fourteen in number. Their number cannot have been less than twelve, since so many are preserved in a single specimen and do not form an entirely unbroken series, and can hardly have exceeded fourteen, because the same specimen has preserved five lumbars, apparently the entire series. It is not impossible, of course, that the typical artiodactyl formula of nineteen thoraco-lumbar vertebræ was exceeded in this genus, but there is no reason to assume that such was the case.

The first thoracic has a short, broad, depressed and distinctly opisthocœlous centrum. The prezygapophyses are like those

of the seventh cervical, but smaller, more concave and without the internal accessory facet. The neural arch is narrower and more strongly convex and the spine is thicker, though of less antero-posterior extent, and inclined backward instead of being erect; the postzygapophyses are on the inferior face of the overhanging neural arch. The pedicels of the arch are very narrow at their point of origin, being deeply notched behind for the passage of the spinal nerves. The transverse processes are long, heavy and prominent and end in large concave facets for the tubercles of the first pair of ribs. Compared with the corresponding vertebra of *Moschus*, the chief differences to be observed consist in the greater breadth of the neural arch and the longer transverse process in the fossil.

The first and second thoracic vertebræ are somewhat different from the others, since they form a transition from the structure of the cervicals. The second has a rather more trihedral and less depressed centrum than the first, a somewhat heavier and longer spine and shorter transverse processes. The prezygapophyses are on the anterior face of the neural arch, but are widely separated. From the second to the sixth thoracic the centra gradually become shorter, less depressed, and more distinctly trihedral in section; the transverse processes shorten and terminate in smaller flat or slightly convex facets for the rib tubercles, instead of concave surfaces; the spines increase in length and the zygapophyses draw near to the median line. The sixth appears to have the longest spine of the series, though this point cannot be determined with certainty. Back of this the spines gradually decrease in height, but still incline strongly backward until the (supposed) eleventh thoracic is reached, which is the anticlinal vertebra. The transverse processes continue long and prominent and still have relatively large rib-facets, but on the eleventh (?) they are greatly reduced and disappear on the twelfth (?). The latter has postzygapophyses of the cylindrical pattern and corresponding anterior processes appear on the thirteenth (?). The centra of the posterior thoracic vertebræ have become considerably elongate; they have subcircular faces, but are constricted and of trihedral section in the middle.

The thoracic region is of about the same length as in *Moschus* and of similar general appearance, but a comparison of the two genera brings to light a number of differences in the details of structure. Thus, in *Protoceras* the transverse processes are decidedly longer, especially in the hinder part of the region. In *Moschus* the spines of the posterior vertebræ are lower, but much more extended from before backward, especially at the tips, which from the tenth to the thirteenth are thickened and project beyond the spine both in front and behind. In this genus also the ninth, tenth and eleventh vertebræ have metapophyses, which are particularly prominent on the tenth and eleventh. In the fossil these arise near the ends of the transverse processes.

The *Lumbar Vertebræ* (Pl. XXI, Fig. 9) numbered at least five. In the anterior region the centra are shaped like those of the posterior thoracic series, but as we pass backward, they become more and more broadened and depressed; the centrum is longest in the second, third and fourth, slightly shorter in the first and considerably so in the fifth. The zygapophyses are of the usual cylindrical, interlocking, artiodactyl type, but no episphenial processes are developed. Large and conspicuous metapophyses are present, which in *Moschus* are hardly more than rudiments, The neural arches and spines are more traguline in shape and appearance than pecoran; the arches are short from before backward, being deeply cleft between the postzygapophyses, which is not the case in *Moschus*, and the pedicels of the arches are not perforated by the spinal nerves (as they are in the modern genus) which pass out through the notches below the postzygapophyses. The spines are less extended antero-posteriorly than in the musk-deer and resemble rather those of *Tragulus*, being slender and curved forward. The transverse processes are likewise quite different from those of *Moschus*. In the latter they are slender and depressed, extending downward as well as outward, and are not at all like the broad, plate-like processes of the higher Pecora. In *Protoceras*, on the other hand, they are long, very broad and thin, rounded at the tip and but slightly decurved, and are thus of the typical pecoran shape. The process is quite short on

the first lumbar, much longer on the second and reaches its maximum length on the third, while the overlapping ilium prevents its attaining any great length on the fifth.

No *Sacral* and but one *Caudal Vertebra* is preserved in any of the specimens. The latter belongs to the proximal part of the tail and has a relatively large subcylindrical centrum, which indicates that *Protoceras* had a much better developed tail than *Moschus* or than the modern deer generally, and which was comparable in proportionate length to that of the giraffe. The prezygapophyses are very long, but do not appear to articulate with any other vertebra. The specimen belongs to a younger and somewhat smaller animal than the skeleton here described and both epiphyses are lost. This fact must be allowed for in using the measurements given below.

The *Ribs.* — In many primitive artiodactyls, such as *Oreodon*, the ribs are notably slender and subcylindrical, resembling those of the carnivores. In *Protoceras* they are entirely pecoran and resemble those of *Moschus* in general, but their somewhat greater length and curvature and the increased distance between the head and tubercle point to a wider and more capacious thorax. The anterior ribs are short, subcylindrical proximally, expanded and plate-like distally; the maximum length is attained about the middle of the thorax; posteriorly the ribs become continually more slender and rounded, as well as shorter.

MEASUREMENTS.

	PROTOCERAS.	MOSCHUS.
Atlas, greatest length	0.050 m.	0.029 m.
Atlas, greatest breadth	.072	.036
Axis, length of centrum	.041	.035
Axis, breadth of anterior face	.032	.024
Axis, length of odontoid process	.013	
Sixth cervical, length of centrum	.025	.025
Sixth cervical, breadth of anterior face	.013	
Seventh cervical, length of centrum	.019	.016
Seventh cervical, breadth of anterior face	.013	
First thoracic, length of centrum	.015	
First thoracic, breadth of anterior face	.015	
Last thoracic, length of centrum	.020	.022
Last thoracic, breadth of anterior face	.018	
First lumbar, length of centrum	.022	.024
Second lumbar, length of centrum	.024	.024

	Protoceras.	Moschus.
Third lumbar, length of centrum	0.024 m.	0.024 m.
Fourth lumbar, length of centrum	.024	.024
Fifth lumbar, length of centrum	.019	.024
Third lumbar, width of transverse process at base	.017	.011
Third lumbar, fore and aft diameter of spine	.011	.021
First caudal, length of centrum	.015	.012
First caudal, breadth of anterior face	.014	.005
First caudal, breadth of posterior face	.013	.004

V. *The Fore Limb.*

The *Scapula* (Pl. XXI, Fig. 10) is entirely ruminant in character and quite different from that of the primitive artiodactyls. In outline it forms a high, narrow triangle, with very slender neck. The glenoid cavity is shallow and nearly circular in shape and the coracoid forms a prominent, recurved hook, which is entirely like that of *Moschus*. The spine is placed near to the anterior margin, so that the prescapular fossa is very much smaller than the postscapular. The spine itself rises rapidly from the suprascapular border and becomes unusually high and prominent; its free border is not, as in most ruminants, an erect edge, but almost from the start is curved over toward the posterior side, the curvature increasing distally. Thus, the hinder surface of the spine is deeply concave and its front convex in a manner resembling the scapular spine of *Mesoreodon*, but unlike that genus, there is no distinct metacromion. The acromion is longer, broader and thicker than in the existing Pecora. The coracoid border is not quite straight, but rises from the neck with a slight curvature, which is concave below and convex above. The other two borders are straight. Both the coracoid and glenoid borders, and especially the latter, are thickened and elevated, making both the pre- and postscapular fossæ somewhat concave. The subscapular fossa is also rendered slightly concave by the elevation of its borders.

The *Humerus* is short and stout. The head is large, strongly convex in the antero-posterior direction and projecting back much beyond the line of the shaft; it is much larger in proportion to the length of the bone than in *Moschus*. The external tuberosity is very large, forming a high, massive ridge,

which extends across the whole anterior face of the bone, is elevated much above the level of the head, and rises toward the inner side, where it forms a blunt, recurved hook, overhanging the bicipital groove. The internal tuberosity is small and laterally compressed, while the bicipital groove is deep and rather narrow. The tuberosities are much more conspicuous than in *Moschus* and (apparently) than in *Gelocus*. The deltoid ridge is but little better developed than in the former genus. The shaft is short and heavy; its promixal portion is laterally compressed, but very thick antero-posteriorly; the middle part is thicker and more rounded in section, and the distal portion is but moderately widened and has a short but quite prominent and rugose supinator ridge. The anconeal fossa is small and of triangular shape; it is considerably lower and broader than in *Gelocus*. The supratrochlear fossa is quite deep, but there is no perforation of the bone at this point.

The trochlea is placed obliquely to the long axis of the shaft, descending somewhat toward the outer side. The intercondylar ridge is a rounded tuberosity, which is much broader and more median in position than in *Gelocus*, though less so than in *Oreodon*, to the humeral trochlea of which that of *Protoceras* bears but little resemblance. The internal epicondyle is much less prominent than in the latter genus, though decidedly more so than in *Gelocus* or the recent ruminants.

The *Ulna* and *Radius* (Pl. XXI, Figs. 11 and 12) are in old individuals coössified at the distal end, but separate for most of their length. The two bones are, however, closely applied together throughout and the radio-cubital arcade is both short and narrow. The ulna is less reduced than in the recent Pecora and the olecranon is higher, straighter, and projects less backward; its free end is thickened and rounded, almost club-shaped, instead of being squarely truncate, and the sulcus along the summit is shallow and obscurely marked. The sigmoid notch has a salient beak and shows a fully differentiated humeral articulation; on the proximal side of the notch the humeral facet extends across the whole width, but distally the facet abruptly contracts and is confined to a narrow strip along the internal border. The shaft of the ulna is much

reduced as compared with that of the oreodonts, suillines, *etc.*, but much less so than in the existing Pecora, or even than in *Leptomeryx* and the tragulines; in its proximal portion, it is convex on the inner, concave on the outer side; distally this arrangement is reversed. The shaft tapers inferiorly, but shows a considerable expansion in the antero-posterior direction about three-fourths of an inch from the distal end, from which it rapidly tapers again. The distal end is relatively much larger than in the recent ruminants and is occupied by a saddle-shaped facet for the cuneiform.

The radius has much the same proportions as in *Moschus*, but is somewhat longer. The head is transversely expanded, particularly toward the ulnar side, and antero-posteriorly compressed; it occupies the entire distal surface of the humerus, the ulna having but a minute facet for this portion of the latter. The proximal surface is divided into three nearly equal facets for the corresponding divisions of the humeral trochlea. The shape of the intercondylar pit of the radius in *Protoceras* is, so far as it goes, a point of resemblance to the oreodonts, but the other humeral facets and the whole shape of the head are entirely different. As these characters are retained with remarkable persistency throughout the whole history of the oreodonts and even in *Agriochœrus*, the difference is not unimportant.

The shaft of the radius is of nearly uniform size throughout, and is strongly arched forward; it is rather slender, but yet of the typical modern ruminant shape, *i.e.*, of transversely oval section, and contrasts strongly with the subcylindrical and remarkably slender radial shaft of *Oreodon*. The distal end is moderately expanded and thickened and bears two roughened, elevated ridges which enclose a sulcus for the extensor tendons. The distal facets for the carpus seem to show some variations which may be more or less due to age. Of the young specimen described by Osborn and Wortman these writers say: "The process of bone which bears this facet (*i.e.*, for the scaphoid) is not produced backward, as it is in *Tragulus*, nor has it the marked obliquity seen in *Leptomeryx* and *Cariacus* and, to a less degree, in *Tragulus*. The scaphoid

facet is not sharply defined by a prominent ridge from that of the lunar, as it is in *Cariacus*, *Leptomeryx*, and *Tragulus*, the two articular surfaces being quite continuous in front" (No. 8, p. 360). In the adult female skeleton which we have been considering, the process of bone bearing the scaphoid facet is produced backward more than in *Tragulus* and almost as much as in *Moschus*, though the lunar does not abut against the ulnar side of the process so extensively as in that genus. The scaphoid facet is distinctly divided from that for the lunar by a ridge which is prominent even in front, though much less so than in the modern ruminants ; it is concave in front and convex behind. The lunar facet is rather narrower than the scaphoidal and is of similar shape, but the posterior convexity is much less extended toward the palmar side. The straight course taken by the facets, to which Osborn and Wortman have called attention, is a difference from all the modern selenodonts, and even in such ancient forms as *Anoplotherium* and *Leptomeryx* the facets run obliquely from before backward and inward. In the camels this obliquity is not very marked. In another respect *Protoceras* differs from all the existing Pecora, Tylopoda, and Tragulina, *viz.*, in the absence of any facet on the radius for the cuneiform.

The manus has already been well described by Osborn and Wortman, but for our present purpose it will be necessary to examine it somewhat more in detail.

The *Carpus* (Pl. XXII, Fig. 17) is remarkably primitive, as compared with the great degree of modernization displayed in the skull. Schlosser (No. 9) long ago called attention to the fact that a constant difference between the more ancient and the later artiodactyls was to be found in the much greater relative height (vertically) of the carpus in the former, especially with regard to the distal elements. This ancient feature is as decidedly marked in *Protoceras* as in the oreodonts. In the shapes of the individual bones, on the other hand, may be seen some approximation to modern conditions. The scaphoid is high, but not much extended transversely or antero-posteriorly. Its shape is thus entirely different from the almost cubical scaphoid of *Oreodon*, and though considerably higher, it

is shaped more as in *Moschus*. The radial facet consists of an anterior convexity, which is lower than in *Oreodon*, and not reflected so far over upon the dorsal face of the bone as it is in that genus, and of a posterior concavity. In the tragulines the scaphoid is relatively much narrower and the anterior convexity for the radius much higher and rising steeply toward the ulnar side, while the inner side is excavated for a descending process of the radius. The proximal surface of the scaphoid is thus much more like that of *Moschus* than of the tragulines and differs markedly from that of *Oreodon* in the sudden narrowing or excavation which invades the posterior concavity from the ulnar side of the bone. The distal end is very different from that seen in any of the groups mentioned above; it is occupied by two distinctly separated facets, for the trapezoid and magnum respectively. The former is rather the smaller of the two and is simply concave and of irregularly oval shape. The magnum facet stands at a somewhat lower level than that for the trapezoid and is nearly flat in front, becoming concave posteriorly to receive the head of the magnum. There is no distinct facet for the trapezium, though Osborn and Wortman state that the two bones are in contact. On the ulnar side are two facets for the lunar, one proximal and one distal, and both nearer to the dorsal than to the palmar border. The distal facets on the scaphoid of *Oreodon* are entirely different from those of *Protoceras* both in shape and in position, which is due to the fact that in the former the magnum has shifted almost entirely beneath the scaphoid, while the lunar has gone over upon the unciform. In the Pecora and Tragulina these facets are changed by the coalescence of the magnum and trapezoid, and in the latter group, by a displacement similar to that which has occurred among the oreodonts. In *Pœbrotherium*, on the other hand, we find a condition very like that of *Protoceras*, with the addition of a minute facet for the trapezium.

The lunar is remarkably high and narrow, narrower even than the scaphoid, which is the reverse of the proportions found in *Moschus* and *Tragulus*, though in *Dorcatherium* (*Hyœmoschus*) the lunar is the narrower of the two. The proximal

end is but little expanded and of nearly uniform width on its dorsal face, though it is somewhat constricted in the middle of the radial side. The radial facet is broader and convex in front, narrower and concave behind. The distal end is described and figured by Osborn and Wortman as having "its articular surface divided almost equally between the unciform and magnum." This description does not quite apply to the adult female skeleton which is here described. While the two facets are not far from being of equal width, a comparison with *Dorcatherium* and *Moschus* shows a tendency toward the traguline method of displacement, in that the facet for the magnum is more lateral and that for the unciform more distal. Consequently, the salient beak formed by the meeting of the two surfaces is not in the vertical median line, as it is in the Pecora, but is shifted toward the radial side. The magnum surface is the narrower of the two, but it extends farther toward the palmar side; it is slightly convex in front and concave behind, while the unciform facet is concave throughout. The scaphoid facets correspond to those already described on that bone. On the palmar side the proximal end of the lunar rises somewhat above the level of the scaphoid and has an accessory lateral facet for the radius, though the contact is less extensive than in recent Pecora. Another accessory radial facet of the proximal end lies behind the principal one. On the ulnar side of the bone are two facets for the cuneiform, the proximal one of which is flat and confined to the dorsal half of the lunar, while the distal one is concave and extends through nearly the entire depth.

The cuneiform is relatively broad, but of proportionately small dorso-palmar depth; its principal difference from that of existing tragulines and Pecora consists in the absence of any facet for the radius. In these groups the displacement of the ulna is to some extent compensated by an extension of the ulnar facet posteriorly and down upon the outer side, but in *Protoceras* the ulnar facet remains a narrow groove. As in the Pecora, the infero-external angle of the bone is slightly incurved to form a blunt hook. The pisiform facet is broad and flat above, narrow and concave below, where it extends

down upon the hook. The distal end is occupied by the wide and shallow (fore and aft) facet for the unciform.

The pisiform is neither traguline nor cervine in character, but differs from that of the musk-deer much as the latter does from the larger species of *Cervus*.

As compared with the pisiform of *Moschus*, it is somewhat longer and more slender and is but slightly incurved at the tip. On the other hand, it differs much more radically from the straight, slender, and elongate pisiform of the oreodonts, and tends distinctly toward the shape taken in the Pecora. It is short, deep, and compressed, rugose and somewhat incurved distally. The facets for the ulna and cuneiform are continuous; the former is small, triangular, and plane, the latter is larger, of irregular outline, and saddle-shaped.

The trapezium is not preserved in any of the specimens in the Princeton collection, but, fortunately, Osborn and Wortman are able to assure us of its presence.

A significant feature in the carpus of *Protoceras*, pointed out by the authors just mentioned, is the complete separation of the magnum and trapezoid, as in *Pœbrotherium* and the Tylopoda generally. The trapezoid is quite high and deep in the dorso-palmar direction, but very narrow; it is somewhat wedge-shaped, broad behind and thinning to an edge antero-externally. Its proximal surface forms a rounded, convex head for the scaphoid, the articular surface of which is continued down upon the palmar side, doubtless for the trapezium. The contact with the magnum is by means of two facets which meet in the middle of the ulnar side. Distally the trapezoid bears a triangular, nearly plane surface for the second metacarpal, but has no contact with the third.

The magnum is relatively high and narrow, as compared with that of the recent Pecora. The dorsal face is subquadrate in outline, the palmar very much narrower, and the posterior hook, though stout and rugose, is very short. The proximal surface is unequally divided between the facets for the scaphoid and lunar. Anteriorly the scaphoid facet takes up quite two-thirds of the width of the magnum and is here slightly concave. Upon the "head" the surface is convex, and,

as the dividing ridge crosses the head obliquely, this region is more equally shared between the two facets. The lunar facet is much narrower than that for the scaphoid ; in front it is more decidedly concave and is lateral rather than proximal in position ; on the head, however, the surface which supports the lunar is truly proximal. On the radial side the small facets for the trapezoid and second metacarpal meet at an open angle and form a salient edge. The distal surface is occupied by a large saddle-shaped facet for the third metacarpal, and rising from this a small one for the second.

The unciform is a large bone, though smaller than the scaphoid, which exceeds it in every dimension save that of thickness ; it is, however, notably high in proportion to its width. The posterior hook, which in the Pecora, including even *Moschus*, is rudimentary, is still well developed, though smaller than in many ancient forms of the Artiodactyla, *e.g.*, *Ancodus*. The proximal surface has two facets, a narrower one for the lunar and a much broader one for the cuneiform. The former is low and slightly concave in front, rising behind into a convex head like that of the magnum. The cuneiform facet is continued down over the ulnar side of the bone, almost reaching the surface for the fifth metacarpal. The magnum surface is confined to a minute strip near the dorsal side, above the facet for the third metacarpal. The latter facet is much more extensive than in those Pecora in which the metacarpals have coalesced to form a cannon-bone and is even larger than in *Gelocus*. The distal surface is taken up by the large facet for the fourth metacarpal ; that for the fifth is very much smaller and rather lateral than distal.

The *Metacarpus* (Pl. XXII, Fig. 17) contains four elements. The median pair (III and IV) have attained about the same stage of development, as regards length and slenderness, as in *Gelocus*, and are therefore relatively longer than in the more ancient artiodactyls, *Xiphodon* excepted. The laterals (II and V) have at the same time retained a size and importance which is utterly unknown in the Pecora and far exceeds what is found in the tragulines. All of the digits remain free throughout life. The mode of connection between the carpus and

metacarpus might with almost equal propriety be called either "unreduced" or "inadaptive," to use Kowalevsky's terms, because this manus is not reduced farther than is involved in the loss of the pollex and enlargement of the median digits. As Osborn and Wortman have suggested, a rudimentary first metacarpal may have been present. This suggestion is made more probable by the fact that the proximal end of metacarpal II has its internal angle truncated and slightly hollowed for the space of about half an inch, as if for the reception of a short splint-bone. This surface is much too large to have been occupied entirely by the trapezium, and nothing of the kind is apparent on mc. V.

The second metacarpal has a narrow, triangular head, with a plane surface for the trapezoid, and on the ulnar side a minute facet for the magnum, which is oblique and rises above the top of mc. III. The small size of this facet as compared with the same in such a genus as *Ancodus*, for example, is an indication that the connection of the magnum with mc. II was undergoing reduction. The shaft is slender, compressed, strongly curved, and of trihedral section, the apex of the triangle being at the contact with mc. III. The distal end is slightly thickened and expanded, but the trochlea is narrow and rounded.

The third metacarpal is the longest of the series, extending both above and below mc. IV, as is also the case in *Oreodon* and *Ancodus*. The head is but little expanded, the longest diameter being the dorso-palmar one, and the head not being extended toward the radial side, as it is in *Gelocus*, in which mc. III covers nearly or quite all the distal surface of the coössified magnum and trapezoid. The proximal surface is taken up by the large, slightly saddle-shaped facet for the magnum, while a stout, oblique process overlaps the head of mc. IV, and abuts against the unciform; this unciform process is longer and more prominent than in *Gelocus*. There is no facet for the trapezoid, mc. III being cut off from that bone by the connection of mc. II with the magnum. On the palmar side of the head is a small circular facet, which projects strongly toward the ulnar side and articulates with a corresponding facet on mc. IV. The shaft is of nearly uniform diameter

throughout, thickening somewhat toward the distal end; in section it is an irregular quadrate, with nearly equal transverse and dorso-palmar diameters, but the palmar surface is much narrower than the dorsal. The ulnar side of the shaft of mc. III and the radial side of mc. IV are flattened, so that the two bones are closely approximated. The distal trochlea is very different from that of *Oreodon* and resembles the pattern found in *Gelocus* and *Pœbrotherium*. In *Oreodon* this structure is low and wide, very strongly convex, and separated from the dorsal surface of the shaft by a deep, narrow pit; while in *Protoceras*, as in the other genera mentioned, it is higher and narrower, the convexity is not so marked, and the articular surface is separated from the face of the shaft only by an almost imperceptible ridge. As in all the genera named, the carina is confined to the palmar side. The radial side of the shaft is flattened for about two-thirds of its length by the contact of mc. II.

Compared with the third metacarpal of *Gelocus* that of *Protoceras* is straighter, with less broadened and thickened head, it has an excavation on its radial side for the head of mc. II. and a larger unciform process; otherwise the two are much alike.

The fourth metacarpal is the counterpart of the third, except that it is shorter and rather more slender proximally, while the distal portion of the shaft is somewhat broader and more compressed antero-posteriorly. The head is no broader than the shaft, but has a greater fore-and-aft diameter, owing to the presence of a palmar projection. The unciform facet is slightly concave transversely. Both of the median metacarpals are proportionately short; while the scapula and the fore-arm bones are considerably longer than in *Moschus*, the median metacarpals are noticeably shorter, and even in the musk-deer the anterior cannon-bone is by no means elongate. The relative length of the metacarpals in *Protoceras* is more nearly that seen in *Dorcatherium*.

Metacarpal V is shorter and distinctly more slender than mc. II, but otherwise shaped like it; the head has a rugosity on the ulnar side for ligamentous attachment. The unciform facet is small, of triangular shape and, but for a small con-

vexity in the middle, is plane; it rises steeply toward the ulnar side, so as to present internally almost as much as proximally.

The *Phalanges* (Pl. XXII, Figs. 17 and 19) are short, both in proportion to the size of the animal and the length of the metacarpus, but are for the most part of typically ruminant pattern, nevertheless. The proximal phalanx is slender and relatively longer than in *Dorcatherium*, though shorter than in *Moschus*. It is nearly straight, and of quite a different shape from the curved, depressed phalanges of *Oreodon*. At the proximal end the dorso-palmar and transverse diameters are nearly equal; the metacarpal facet is not concave and only the palmar border is notched for the metacarpal carina. The distal end is depressed, wider than deep, and its trochlea is obscurely divided by a shallow notch into two condyles.

The second phalanx is notably short and rather broad and depressed, the transverse diameter exceeding the dorso-palmar. This phalanx is thus of quite a different shape from that which characterizes the recent chevrotains and Pecora. The distal trochlea is neither deeply grooved nor reflected far upon the dorsal side of the bone.

The ungual phalanx is altogether ruminant in character, its shape being trihedral, slender and pointed; it is not so slender and elongate as in *Moschus*, nor so curved and depressed as in the tragulines, and is less symmetrical than in *Pœbrotherium*, indicating that the median digits were more closely approximated than in that genus.

MEASUREMENTS.

	PROTOCERAS No. 1.	PROTOCERAS No. 3.[1]	MOSCHUS.
Scapula, height	0.131		0.106
Scapula, greatest breadth	.069		.059
Scapula, breadth of neck	.015		.014
Scapula, fore-and-aft diameter of glenoid cavity	.022		.018
Scapula, transverse diameter of glenoid cavity	.018		.015
Humerus, length (fr. ext. tub.)		.149	.149
Humerus, thickness of proximal end (ant.-post.)		.045	.032
Humerus, breadth of distal trochlea		.025	.019
Radius, length	.147	.140	.127

[1] This individual is part of the type specimen of *P. celer* Marsh; it was found in 1894 by Mr. Hatcher.

	PROTOCERAS No. 1.	PROTOCERAS No. 3.	MOSCHUS.
Radius, breadth of proximal end	.025	.026	.019
Radius, breadth of distal end	.015	.016	.019
Radius, breadth of shaft in middle	.015		.011
Ulna, length	.182		.154
Ulna, height of olecranon	.033		
Ulna, breadth of distal end	.009	.010	.007
Carpus, height	.021		.014
Carpus, breadth	.023		.018
Magnum, height of dorsal face	.008		.004
Metacarpal II, length	.081		.027
Metacarpal II, breadth of proximal end	.007		
Metacarpal II, breadth of distal end	.008		.005
Metacarpal III, length	.089		.102
Metacarpal III, breadth of proximal end	.011	.012	.010
Metacarpal III, breadth of distal end	.010		.010
Metacarpal IV, length	.085		.100
Metacarpal IV, breadth of proximal end	.008	.010	.010
Metacarpal IV, breadth of distal end	.010	.010	.010
Metacarpal V, length	.077		.034
Metacarpal V, breadth of proximal end	.006		
Metacarpal V, breadth of distal end	.006		.005
First phalanx, III digit, length	.024	.022	.028
First phalanx, III digit, breadth of proximal end	.010	.010	.010
First phalanx, III digit, thickness of distal end	.006	.007	.006
Second phalanx, III digit, length	.012		.021
Second phalanx, III digit, breadth of proximal end	.009		.007
Second phalanx, III digit, thickness of distal end	.007		.007
Third phalanx, III digit, length	.017		.021
Third phalanx, III digit, breadth of proximal end	.009		.008
Third phalanx, III digit, thickness of proximal end	.009		.008

VI. *The Hind Limb.*

The hind leg is much longer and heavier than the fore leg in all its parts. The disproportion is, however, by no means so great as in *Tragulus*, but rather resembles the condition found in *Moschus*. In the living animal the increased length of the hind leg was compensated by the greater habitual flexure of the knee and hock joints.

The *Pelvis* is pecoran rather than traguline in character. The ilium has a thin and compressed, but rather short and deep, plate-like neck, which expands gradually into the large and everted anterior portion. The outline of the anterior or supra-iliac border is like that of *Cervus* more than of *Moschus*

in having distinct upper and lower projections. The sacral articulation is placed unusually far back, nearly the whole of the anterior expansion being in front of it. The acetabular and pubic borders are quite widely separated at their points of origin, but approximate forward; and the anterior part of the iliac surface is a narrow groove. The ischial border, which in *Tragulus* is nearly straight, is arched upward above the acetabulum into a crest, as in the Pecora. The fossa in front of the acetabulum and above the acetabular border is not so large or so deep as in *Moschus;* in *Tragulus* it is wanting. The acetabulum is small, nearly circular in outline, and deep; the fossa for the ligamentum teres encroaching but little upon the articular surface. The ischium is long, deep vertically, laterally compressed and plate-like in form. At the posterior end it is considerably expanded, the dorsal border rising more gradually and not forming an overhanging hook, as in *Moschus*. The tuberosity is rather small, but prominent and rugose and situated higher up than in the recent genus. The pubis at its point of origin is slender and of a rounded, depressed section; but it soon expands and becomes plate-like, as in the Pecora, not having the rod-like character found in the Tragulina. The obturator foramen is a long, narrow oval, considerably more elongate than in *Moschus*. As a whole, the pelvis is thus distinctly pecoran in type.

The *Femur* (Pl. XXII, Figs. 13 and 14) is much like that of *Moschus*, though not without some considerable differences. Thus, the head is less distinctly set upon a neck and is ovoidal rather than hemispherical in shape. The great trochanter is broader, more massive, and rises higher above the level of the head; the deep digital fossa does not extend so far behind the head and the ridge connecting the great trochanter with the second is much more prominent. The second trochanter forms a large, rugose, pyramidal protuberance. These differences, it is obvious, are mere matters of detail; and the proximal end of the femur is much more like that of *Moschus* than that of *Tragulus*. A resemblance to the latter is seen in the shaft, which is heavier than in *Moschus*, as well as longer, but has a similar rounded shape and strong anterior curvature,

and the external linea aspera is much better marked and longer. The pit for the attachment of the plantaris muscle is larger and deeper, but has not such a rugose bottom and is not so conspicuous when viewed from the side, which is due to the fact that in the musk-deer the outer wall of the pit is cut away. The rotular groove is very different from that of *Moschus*. In the latter the groove is narrow, symmetrical, with borders of equal height and thickness, and continued well up upon the anterior face of the shaft, while in *Protoceras* we find a condition more like that of the larger deer, though not in the same degree of development. In this genus the trochlea is broad and shallow; the inner border is considerably higher and thicker than the outer and the articular surface is reflected over upon the mesial face of the bone; but there is no such vertical prolongation of the groove upon the shaft. The characteristic rotular trochlea of the tragulines, which is also found in *Leptomeryx*, is quite different from that of *Protoceras*, which in this respect approximates more to the higher Pecora. The condyles are narrow and project less behind the plane of the shaft than in *Moschus*, more than in *Tragulus*.

The *Patella* (Pl. XXII, Fig. 15) is remarkably large in proportion and somewhat peculiar. It is of the same general shape as in the musk-deer, broad above and tapering below to a blunt point, but is more massive and rugose and larger in every dimension. The fossa for the inner border of the femoral rotular groove is more deeply impressed than that for the outer border, and its internal edge forms a prominent projection, which extends along the mesial face of the femur. There is considerable individual or perhaps specific variation in the shape of the patella. One specimen is broader and less tapering than the one above described, and its inner prominence is not so much produced.

Both specimens of the *Tibia* (Pl. XXII, Fig. 16) in the collection have lost the distal end, but fortunately this is preserved in Osborn and Wortman's material. Though longer and heavier than the tibia of *Moschus*, that of *Protoceras* is of essentially the same character. The condyles for the femur are rather narrow, but well extended from before backward;

the spine is bifid and unusually high, but this may in part be due to the crushing which the specimens have undergone. The cnemial crest is very prominent, ends above in a massive rugosity and the sulcus for the peroneal tendon is deeply incised. The shaft has the characteristic double curvature, both anteriorly and laterally, and the general shape found in the smaller Pecora. According to Osborn and Wortman, the astragalar surface is constituted very much as in the deer (*Cariacus*).

The proximal portion of the *Fibula* is coössified with the tibia, as in *Moschus*, *Tragulus*, etc., and as this portion is considerably thicker proportionately than in the genera named, it is likely that a good length of the slender, filiform shaft was preserved in the living animal. Osborn and Wortman state that the distal end forms a distinct malleolar bone, which is shaped as in the Pecora and wedged in between the calcaneum and the distal end of the tibia. They find reason to believe, however, that in the fully adult animal the two bones may coalesce, as in the tragulines. In the latter group the malleolar bone, even when separate from the tibia, as according to Flower it sometimes is in *Dorcatherium*, has quite a different shape from that of the Pecora. The tibia and fibula of *Leptomeryx* are remarkably like those of *Protoceras* in almost every respect.

The hind foot has already been described by Osborn and Wortman, but it will be necessary to go over the same ground with somewhat more fullness, in order to display the range of variability in this structure, and to bring together all the material for comparison in attempting to estimate the systematic position of this extraordinary genus.

The *Tarsus* (Pl. XXII, Fig. 18) unites a condition of primitiveness with highly advanced characters. The calcaneum is thoroughly pecoran in shape; the tuber is quite elongate (more so than in *Moschus*, less than in *Cervus* or *Gelocus*), compressed, with nearly parallel dorsal and plantar borders, tapering less to the distal end than in the musk-deer, and with less definitely marked tendinal sulcus on the free end. The sustentaculum is very prominent and at once distinguishes this calcaneum from that of any of the oreodonts. The fibular facet is not so

much elevated as in *Moschus* or *Cervus*, but is longer antero-posteriorly; the inner surface of this prominence bears a facet for the astragalus. The distal astragalar facet forms a broad, flat band, which is connected with the cuboid facet. The latter surface is relatively broader than in the Pecora, but has a much greater dorso-plantar extent than in *Moschus*, the calcaneum not being so suddenly constricted distally as in that genus.

The astragalus is higher and narrower than in recent Pecora, a primitive character which is repeated in such genera as *Gelocus*, *Pœbrotherium*, *etc.* The proximal trochlea is widely and deeply grooved, and the external condyle is slightly higher and thicker than the internal, and is separated from the corresponding distal surface by a much wider interval than in the modern deer; the sustentacular facet is also relatively narrower than in those animals. The distal astragalar trochlea is not only higher and narrower than in the existing Pecora, but is also differently proportioned. The cuboidal surface, in the first place, is distinctly narrower in relation to that for the navicular. In the second place, the junction of the two facets is marked by quite a prominent angulation, while in the modern forms this is a low, rounded swelling. *Pœbrotherium* again agrees with *Protoceras* in this respect, though the cuboidal facet is broader in the former.

There would appear to be considerable variation with regard to the coössification of the various tarsal elements. Osborn and Wortman say: "In our young specimen of *Protoceras* the cuboid and navicular are perfectly free, but in the adult specimen there is some bony union. The line of junction, however, is clearly indicated by a more or less open suture. What is here said of the cuboid and navicular also applies to the cuboid and ectocuneiform, so far at least as the union of the latter with the cuboid is concerned. There appears to be no tendency to bony union of the ectocuneiform with the navicular." In the Princeton collection are feet belonging to individuals which are not only adult but aged, and in none of them is there any ankylosis of the cuboid with the navicular or of the ectocuneiform with either.

Like the astragalus, the cuboid is higher than in the recent Pecora; the calcaneal surface is relatively wider, the astragalar facet narrower than in those animals. The calcaneal facet is wider than the corresponding distal portion of the bone and hence forms an overhanging ledge. The astragalar surface is narrow and simply concave in the dorso-plantar direction; shortly behind the points where the two facets join they are narrowed by a circular sulcus and another invades the astragalar facets of both cuboid and navicular. The principal diameter of the cuboid is the dorso-plantar, due partly to the large size of the posterior hook, which is more or less rudimentary in the modern Pecora. Of the inner or tibial side of the cuboid about one half the height is occupied by the navicular, which is supported upon two narrow, ledge-like projections, the posterior one of which is considerably more prominent than the anterior. Below the ledge on the dorsal side is a flat facet for the ectocuneiform and there is an additional facet for this bone in the middle of the cuboid. The distal surface is occupied by the large and somewhat irregular facet for the fourth metatarsal, which is convex in front and concave behind. The facet for the fifth metatarsal is very small and entirely lateral in position, much as in *Pœbrotherium*. An additional facet for the plantar projection of mt. IV is formed on the infero-internal side of the cuboid hook.

The navicular is higher than in the recent Pecora, but not otherwise notably different. The astragalar surface is concave, but has a low, rounded ridge near the fibular border for the groove on the distal trochlea of the astragalus. On the proximal dorsal margin this ridge forms an elevation which is less distinctly marked than in most of the existing Pecora. The portion of the circular sulcus above mentioned as invading the approximate astragalar surfaces of the cuboid and navicular, which affects the latter, forms a channel or groove along the whole fibular side of the bone. On the same side are two well defined facets for the cuboid, of which that on the plantar side is the larger and presents more inferiorly. On the distal side are two very distinctly separated facets for the cuneiforms. The anterior one, which is for the coalesced ecto- and meso-

cuneiforms, is L-shaped, occupying the dorsal and tibial sides of the bone. The surface for the entocuneiform is relatively very large in the dorso-plantar direction, but very narrow transversely; it is placed on a downward projection from the distal face of the navicular and stands at a considerably lower level than the facet for the compound bone. The posterior hook from the plantar side of the navicular, which is so conspicuous in the oreodonts, is absent in *Protoceras*.

As in nearly all known selenodonts, recent or fossil, the meso- and ectocuneiforms are indistinguishably fused together. This occurs even in the oldest known American artiodactyl, *Trigonolestes*, of the Wasatch. The compound element thus formed is nearly as broad as the cuboid, and of much greater vertical height relatively than in the recent Pecora; it articulates with the second and third metatarsals.

The entocuneiform is much larger proportionately than in the recent Pecora or Tragulina; its principal dimension is the vertical one and it forms a high, deep, and compressed plate, which has numerous connections. Proximally it articulates with the navicular and distally with the head of the second metatarsal, also with the projection from the plantar side of the third. In the Pecora this latter articulation does not exist.

The *Metatarsus* (Pl. XXII, Fig. 18) consists of four elements, two of which (mt. II and V) are splint bones and two (III and IV) are large functional digits. In one of the specimens described by Osborn and Wortman the second metatarsal is more than one-third the length of the functional pair, but ordinarily it is much shorter and forms a narrow compressed splint, tapering to a point inferiorly. It articulates with the mesocuneiform portion of the compound element and by a long truncated surface with the entocuneiform.

The third metatarsal is very much larger, longer, and heavier than the corresponding metacarpal. The head bears a large, somewhat concave facet for the compound cuneiform; the posterior hook-like projection, which in existing Pecora has become rudimentary, is very large and prominent and bears an oblique facet for the entocuneiform, as is also the case in *Oreodon*. On the tibial side of the head is a deep fossa in

which the proximal part of mt. II lies and on the fibular side is a narrow, concave facet into which a projection from mt. IV is received. The shaft is stout, slightly contracted in the middle; proximally it is laterally compressed and has its greatest diameter in the dorso-plantar direction, while distally it is broadened and flattened and its longest diameter is transverse. This is in accordance with the shape of the metatarsal in the Pecora, in which the hind cannon-bone is laterally compressed in its proximal portion, while the fore cannon-bone is compressed from before backward, but in *Protoceras* the difference is not so distinctly marked. The contact surfaces of the median metatarsals are flattened, allowing the two to be very closely approximated; the plantar side is also flattened, but the dorsal and apposite surfaces form one continuous curve. The distal trochlea is strongly convex and is demarcated from the shaft by a shallow pit; the carina, as in the metacarpal, is confined to the plantar side, but is a little more strongly developed than in the manus.

The fourth metatarsal is the counterpart of the third and exceeds it but very slightly in length; it projects somewhat below the distal end of mt. III, but this is nearly compensated by the fact that the head of the latter rises a little above that of mt. IV. The plantar hook is very prominent and bears a facet for the one upon the hook of the cuboid. The fibular side is excavated for the head of mt. V, and there is a minute articular surface for that bone; the fossa, however, is not nearly so deep and wide as that on mt. III, which receives mt. II. The articulation with mt. III is by means of a small but prominent process on the dorsal margin of the proximal end, which fits into a corresponding depression on mt. III, and also by flat surfaces on the approximate sides of the plantar hooks. According to Osborn and Wortman, some specimens show a tendency to the ankylosis of the median metatarsals; but none of those in the Princeton collection, even of aged individuals, exhibit any traces of such a process, and it would appear to be very exceptional.

The fifth metatarsal is a short, tapering splint; it articulates with a small facet on the fibular side of the cuboid and with

another and still smaller one on the head of mt. IV. No traces of any distal portions of the metatarsals of the lateral digits, such as Kowalevsky figures for *Gelocus*, have yet been found, nor is there any reason to believe that such existed. Indeed, Kowalevsky does not explicitly state whether they are figured in the pes on any better evidence than the analogy of the manus.

The *Phalanges* (Pl. XXII, Figs. 18, 20), so far as is known, are confined to the median digits, and in all probability, there were no dew-claws. The phalanges of the pes are very much larger than those of the manus and differ from them in several details of structure, indeed, to quite an unusual degree. Aside from its great increase in every dimension, the proximal phalanx is like the corresponding bone in the manus; the distal trochlea is somewhat more deeply notched in the median line and has a relatively greater dorso-plantar diameter, while on the plantar side the trochlea is more prominent and continued farther proximally upon the shaft.

The second phalanx is not only actually, but also proportionately, much longer than the corresponding anterior phalanx, and is of quite a different shape, being laterally compressed and having its principal diameter the dorso-plantar instead of the transverse. The proximal articular surface is more deeply concave, more distinctly divided into two cotyles by a median ridge, and the median elevation of the dorsal margin is more pronounced. The rugose prominences for ligamentous attachment are larger and more unsymmetrical, that on the external side (tibial side of digit III and fibular side of digit IV) being decidedly the more prominent. The distal trochlea describes an almost exact semicircle and is extended more proximally upon the dorsal side than is the case in the manus.

The ungual phalanx is longer and straighter than the anterior one, its outer border being less curved, and the distal end is more obtusely pointed; the plantar face is more concave, with more elevated borders, and the rugosity on the plantar side beneath the articular facet is much more prominent. The facet for the second phalanx is higher, narrower, and more symmetrical, in that its two divisions are more nearly of

the same size. The dorsal border of the facet is prolonged in the median line into a large overhanging hook, which is but slightly indicated in the anterior ungual. This hook corresponds to the greater dorso-plantar diameter of the distal trochlea of the second phalanx and its greater prolongation up the dorsal face of the shaft in the pes than in the manus.

As will be seen on comparison of the tables of measurements, there is not in *Moschus* such a difference between the anterior and posterior phalanges as obtains in *Protoceras*. Those of the pes are of nearly the same size in both genera, though slightly larger in *Protoceras*, while those of the manus are considerably larger in *Moschus*. In structure also the posterior phalanges agree better in the two animals, the differences being merely in matters of minute detail, except, of course, the grooves for the metatarsal keels.

MEASUREMENTS.

	PROTOCERAS.	MOSCHUS.
Ilium, length of ventral border (fr. acetabulum)	0.116	0.069
Ilium, depth of neck	.023	.011
Acetabulum, fore and aft diameter	.024	.020
Acetabulum, vertical diameter	.020	.019
Femur, length	.177	.164
Femur, breadth of proximal end	.042	.035
Femur, breadth of distal end	.041	.032
Patella, height	.036	.025
Patella, breadth	.024	.013
Tibia, breadth of proximal end	.039	.035
Tibia, thickness of proximal end	.046	.037
Calcaneum, length	.070	.051
Astragalus, length	.030	.022
Astragalus, breadth of proximal trochlea	.016	.015
Cuboid, height	.016	.008
Cuboid, breadth of distal end	.011	.008
Metatarsal III, length	.107	.131
Metatarsal III, breadth of proximal end	.012	.007
Metatarsal III, breadth of distal end	.013	.009
Metatarsal IV, length	.107	.134
Metatarsal IV, breadth of proximal end	.011	.008
Metatarsal IV, breadth of distal end	.012	.009
First phalanx, III digit, length	.035	.031
First phalanx, III digit, breadth of proximal end	.013	.010
First phalanx, III digit, thickness of distal end	.009	.009
Second phalanx, III digit, length	.022	.023

	PROTOCERAS.	MOSCHUS.
Second phalanx, III digit, breadth of proximal end	.011	.010
Second phalanx, III digit, thickness of distal end	.011	.008
Third phalanx, III digit, length	.023	.024
Third phalanx, III digit, breadth of proximal end	.010	.008
Third phalanx, III digit, thickness of proximal end	.016	.011

VII. *Restoration* (Pl. XX).

The general aspect of the skeleton, as a whole, resembles that of the musk-deer. The head is proportionately much longer, a character frequently found in the ancient mammals, as compared with their recent representatives, though in this case the elongation principally affects the facial region, which is not an ancient but a modern character. In the living animal the appearance of the head must have been entirely different from that of *Moschus;* even the female doubtless had the proboscidiform muzzle, which among the recent ruminants is found only in the saiga antelope and to a less extent in the moose (*Alces*). The difference is, of course, exaggerated in the case of the male, whose bizarre skull is not to be compared with that of any existing mammal whatever.

The spinal column, in length, weight, curvature, and in the proportions of the various regions, is very similar indeed to that of *Moschus;* the neck is heavier and actually longer, though much shorter as compared with the length of the skull; the trunk is of about the same length and the thorax deeper and more capacious. The lumbar region has broader transverse processes, but more delicate spines, which curve more decidedly forward, and more prominent metapophyses, all of which are traguline features and perhaps indicate a more pronounced curvature of this region of the back than in the musks. There can hardly be any doubt that *Protoceras* had a distinctly longer and better developed tail than most recent deer.

The inequality in the length of the fore and hind limbs is very nearly the same as that which is to be observed in *Moschus*, but the proportions of the different limb-segments are not similar and the individual bones are heavier and stronger. Thus, the scapula and the bones of the fore-arm are considerably longer than in the existing animal and the carpus is much

higher; but, on the other hand, the metacarpus and anterior phalanges are very much shorter and the humerus is of similar length in the two genera. In the hind limb similar facts are observable; the pelvis, femur, tibia, and tarsus are all decidedly longer than in the musk-deer, while the metatarsals are much shorter. The phalanges of the pes are of nearly the same length in both genera. As a whole, the limbs are longer in *Protoceras*, the shortness of the feet not compensating for the greater length of the other segments of the limbs.

The skeleton of *Leptomeryx* follows that of the tragulines more closely than it does that of *Protoceras*, both in regard to the actual size of the body and in the relative length of the limbs and consequent curvature of the back. The inequality of the limbs and curvature of the spinal column are, however, decidedly less than in *Tragulus*. Aside from the great difference of stature and the still greater divergence in the appearance and character of the skull, there is an undeniable resemblance between the skeletons of *Leptomeryx* and *Protoceras*, though that between the latter and *Moschus* is still closer.

VIII. *The Systematic Position of Protoceras.*

The osteology of this genus is now almost as completely known as that of any living mammal, and yet the determination of its affinities is a very obscure and difficult problem, and it is therefore hardly a matter of surprise that there should be much difference of opinion with regard to it. Marsh cautiously infers from an examination of the skull alone that it was connected with the giraffe. "The characters now known suggest affinities with the giraffes, but indicate a distinct family." (No. 5, p. 82.) Zittel makes the genus the type of a subdivision of his family "Cervicornia," and says of it: "Die Gattung *Protoceras* bildet ein höchst merkwürdiges Bindeglied zwischen *Tragulina* und *Cervicornia*. Gebiss und Extremitäten stimmen mehr mit den ersteren überein, während sich der Schädel am besten mit den Giraffen und Sivatheriden vergleichen lässt." (No. 11, p. 407.) Osborn and Wortman express no very decided opinion as to the relationships of *Protoceras*, leaving the

question rather an open one. "If now we compare *Protoceras* with any family of the Pecora, there are so many striking differences at once apparent that we are compelled to conclude that there are no marked affinities in the direction of any of these families. In the possession of bony protuberances on the parietals, which are probably processes of this bone, and not developed separately as in the Giraffe, in the general architecture of the skull, together with so many primitive characters of the feet, this genus apparently occupies a distinct position and cannot be consistently referred to either the Tragulina or the Pecora as at present constituted and defined. The possession of multiple horns suggests the possible relationship of this family to the Sivatheriidæ, but the likeness does not extend to other features of the skull." "That it [*i.e.*, *Protoceras*] represents a distinct family there can be little doubt. Of its successors we know nothing whatever, and our ignorance is equally great in the matter of its ancestry." (No. 8, p. 369.)

Flower has well stated the difficulty of determining the relationships of artiodactyl groups, the ancestry of which can only be conjectured. "The *Pecora* or true Ruminants form, as has often been remarked, an extremely homogeneous group, one of the best defined and closely united of any of the Mammalia. But though the original or common type has never been departed from in essentials, variation has been very active among them within certain limits, and the great difficulty of subdividing them into natural groups ('the despair of zoölogists,' as Pucheron calls it) arises from the fact that the changes in different organs (feet, skull, frontal appendages, teeth, cutaneous glands, etc.) have proceeded with such apparent irregularity and absence of correlation, that the various modifications of these parts are most variously combined in different members of the group." (No. 2, p. 181.) All this applies almost equally well to the Artiodactyla as a whole, and the mutual relationships of the various subdivisions which compose that order. This difficulty proceeds from the frequent impossibility of determining what points of resemblance between the groups to be compared are due to inheritance from a common ancestor, and what are cases of parallel development. Such determination

can be made with certainty only when the phyletic series has been worked out, and here, as elsewhere, any classification which is made without knowledge of the various phyletic steps, can be only temporary and tentative.

In the case of *Protoceras* we may hope, with the complete information as to its structure which we possess, to establish its relationship to the Pecora as a whole with reasonable probability, the main outlines of the latter's phylogeny having been fairly well determined. We shall need to remember Osborn's dictum in this discussion, that "no case of exact parallelism in both teeth and feet between two unrelated types has yet been found, or is likely to be." (No. 7, p. 383.) If the European palæontologists, beginning with Kowalevsky, are right, then the ancestral form of the modern Pecora is the Oligocene genus, *Gelocus*, and no fact is known which in any way impugns the probability of this conclusion. The first step in our inquiry must therefore be to institute a comparison between *Gelocus* and *Protoceras*. If the latter be referable to the Pecora at all, it must be derived from *Gelocus*, which there is good reason to regard as ancestral to that entire group.

So far as the dentition is concerned, there is no very essential difference between the two genera, but the European form has molars in a less advanced stage of development, as is to be seen in the thicker, more conical, and less completely crescentic form of the lobes. The premolars are likewise less differentiated. The upper canine is compressed and blade-like, while in *Protoceras* it is trihedral and opposes the first lower premolar. The skull of the American genus is in many respects much more modernized than that of *Gelocus*, though the latter is only imperfectly known. Kowalevsky says of it: "Leider habe ich in allen untersuchten Sammlungen keinen completen Schädel finden können. Indess aus verschiedenen Bruchstücken des Schädels geht unzweifelhaft hervor, dass Gelocus weder geweihartige Auswüchse, noch Hörner auf den Stirnbeinen besass. Dieselben Bruchstücke haben gezeigt, dass die eigentliche Hirnkapsel nicht so weit nach hinten verdrängt war, wie es bei den heutigen Wiederkäuern der Fall ist, sondern eine mehr normale Stellung einnahm, in der Weise,

dass der vordere Orbitalrand sich genau dem ersten Molar gegenüber befand, während bei dem grössten Theil der recenten Wiederkäuer die Hirnkapsel, in Folge der starken Entwickelung des Gesichtstheils, so weit nach hinten verschoben erscheint, dass der vordere Orbitalrand dem letzten Molar gegenüber, oder selbst hinter diesen zu stehen kommt. Der Schädel hatte eine gewisse Aehnlichkeit mit dem unserer heutigen Traguliden, mit denen Gelocus überhaupt viele gemeinsame Merkmale besitzt." (No. 3, p. 147.)

In sharp contrast with the primitive traguline skull of *Gelocus* is the advanced and highly differentiated skull of *Protoceras*, which in some respects is more modernized than that of the deer. The short, capacious, and rounded cranium, the posterior position of the orbits, the great elongation of the face and its depression upon the basicranial axis are characters which at present are confined to the higher Pecora. At the same time, the shape of the occiput, the arrangement of the supraoccipital, the prominence of the sagittal and lambdoidal crests are primitive features, such as are now not to be found in even the lowest Pecora, *e.g.*, *Moschus*. The specializations peculiar to this skull form still a third class of characters, such as the extreme shortening of the nasals and the numerous protuberances which are developed in the male, especially those which arise from the parietals and maxillaries. The skull of *Protoceras* presents, then, a remarkable assemblage of characters, some few even more primitive than are found in the tragulines, some altogether peculiar to itself, but most of them extremely modernized and advanced, and elsewhere among selenodonts combined only in the higher Pecora. Though an examination of the skull alone might lead one to refer this genus to the Pecora, yet there would be much reason to hesitate in doing so. The very combination of such primitive and modern characters is not what we should expect in a transition form ; in such a form we should find an association of features like those of *Tragulus* and *Gelocus*, on the one hand, and of the lower Pecora, such as *Moschus*, on the other. Then, too, the existence of horn-like protuberances on the *parietals* is not suggestive of relationship with the Pecora, since no member

of that group has any such protuberances, their horns and antlers always rising from the frontals.

The other parts of the skeleton bear testimony of similar import. The atlas and axis do not differ in any important respect from those of *Gelocus*, so far as the latter are known. In particular, the odontoid process has attained the same stage in the development of the spout-like shape. The remaining cervical and the thoracic vertebræ are very similar to those of the musk-deer, while the lumbars are more traguline.

The limbs are in almost every respect far more primitive than those of *Gelocus*. The scapula, however, has a more modernized form than in that genus, and except for its more prominent and recurved spine, is a copy of that of the musk-deer ; while in *Gelocus* it is wider than in either the recent tragulines or true ruminants. The fore-arm bones are decidedly less advanced than in *Gelocus*. In the latter the groove on the proximal end of the radius for the intercondylar ridge of the humerus is much narrower, the distal facets for the carpus are much more oblique in position, and there is an articulation with the cuneiform. The shaft of the ulna is much more reduced, and its distal end no longer covers the entire cuneiform. The carpus of *Gelocus* shows a great advance over that of *Protoceras*, both in its reduced vertical height and in the ankylosis of the trapezoid and magnum. In the metacarpus the same advance is visible in the reduction of the lateral digits to splint-bones, which are interrupted in the middle, in the exclusion of the second metacarpal from contact with the magnum, and the articulation of the third with the trapezoid. In all these respects *Protoceras* is very much more primitive. Filhol adds another modernization of *Gelocus*, *viz.*, the frequent coössification of the proximal parts of the lateral metacarpals with the median pair, although the latter are not themselves ankylosed to form a cannon-bone.

Femur, tibia, and fibula show no important differences in the two genera, but the pes of *Gelocus* is much more modernized. This advance consists in the coössification of the cuboid and navicular and the coalescence of the third and fourth metatarsals into a cannon-bone, as well as in the reduced

height of the distal tarsals. If Kowalevsky's figure of the pes be correct, *Gelocus* probably is behind *Protoceras* in the retention of the distal portion of the lateral metatarsals. However, neither this writer nor Filhol speaks of finding these portions *in situ*, and, if their association with the pes is conjectural, no great stress can be laid upon this character.

The result of this comparison, then, is that in regard to the structure of the skull, and to a less degree the dentition, *Protoceras* is far in advance of *Gelocus*, while the differentiation of the limbs, and especially of the feet, lags as far behind. This association of characters confronts us with very clearly defined alternatives, when we attempt to solve the problem of the relationship of *Protoceras* to the Pecora. Assuming, as we have every justification in doing, that *Gelocus* is an ancestral form of the Pecora, then, either *Protoceras* is not descended from *Gelocus* at all, and its likenesses to the Pecora are not due to genetic affinity, but have been independently acquired; or, on the other hand, *Protoceras* is so descended, its resemblances to the true ruminants are the expression of real relationship and the primitive structure of the limbs and feet has been reacquired, whether by reversion or otherwise. Of these alternatives the former is by far the more probable. (1) Without at all denying the *possibility* of such reacquisition of primitive characters, yet no plausible reason can be assigned for assuming it, and no case is known, among mammals at least, in which such a mode of development has been rendered in the smallest degree likely. (2) On the other hand, very many cases of the independent acquisition of similar structures have been pretty clearly demonstrated. It will suffice to mention the many different groups which have independently developed the spout-shaped odontoid process of the axis, or the tetraselenodont molar, or the humerus of the horse and camel. It may, perhaps, be objected that these are single characters, whereas the skull of *Protoceras* displays a whole series of such resemblances to the Pecora, but even such cases of parallelism are by no means unknown. There are many close correspondences in the skull-structure (and limb-structure as well) between the camels and the true ruminants, and yet the suc-

cessive genera of these two series show that these resemblances are not due to a common ancestry, since there can hardly have been an ancestor common to both series later than *Dichobune*, or some similar form. Even more remote is the connection between the true ruminants and the true swine (*Suidæ*), yet the latter have acquired several of those very characters which give such a strong pecoran stamp to the skull of *Protoceras*, for example, the backward shifting of the orbits and the depression of the face upon the basicranial axis. In the loss of the sagittal crest the swine-skull is more modernized than that of *Protoceras*. Among the oreodonts, *Merycochœrus* has the face bent down upon the cranial axis, and the orbits are shifted much farther back than in any other member of that family. Such resemblances are obviously, therefore, not sufficient in themselves to create any strong presumption of affinity. The foot-structure, by keeping on such a primitive plane, reveals the true nature of these skull-characters. As Osborn has said, we know of no case where teeth, skull, and feet have converged to a common type from different starting-points, but that one of these may display such convergence or parallelism in several different respects is not at all uncommon.

(3) Even should we go so far as altogether to exclude *Gelocus* from the pecoran ancestry, the difficulty of accounting for the peculiar assemblage of characters found in the skull of *Protoceras* on any other hypothesis than that of the independent acquisition of the pecoran features, is not at all diminished. For it must be remembered that these likenesses are to the higher members of the group, and on the same grounds such typical members of the series as *Prodremotherium*, *Dremotherium*, *Amphitragulus* and *Palæomeryx* would have to be excluded from the line.

It is altogether probable, then, that *Protoceras* has but a remote connection with the Pecora, and consequently that the affinities with different families in that group which have been suggested, are illusory. Nor can it properly be called a connecting link between the tragulines and the deer, for, having more primitive feet than the former group, it cannot well be

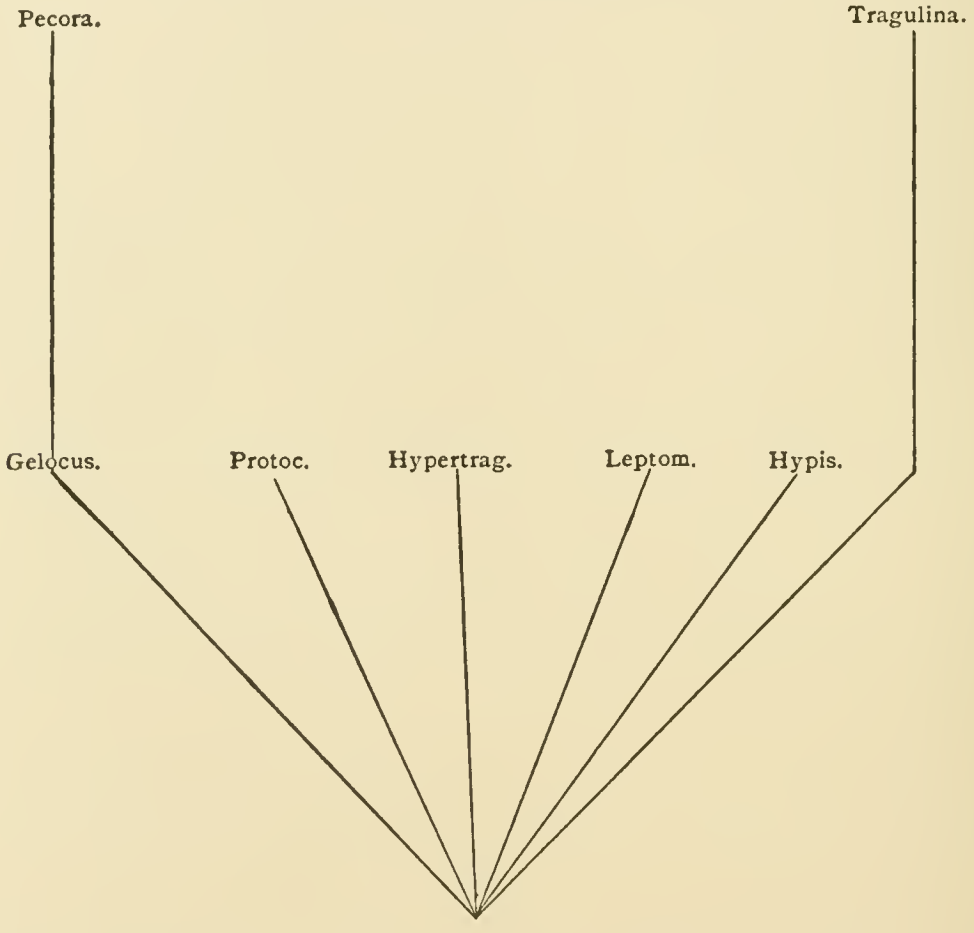
descended from it, and the skull being in some respects more advanced than that of the deer, *Protoceras* can hardly be ancestral to that family.

It is not at all likely that the Tragulina are descended from *Gelocus*, the latter having a more advanced type of foot-structure and an odontoid process of the axis which has already lost the conical shape. The connection with the true ruminants was, therefore, in all probability by means of some form as yet unknown, which was rather more generalized than *Gelocus*. With the line terminating in the existing tragulines, which, so far as we know, have always been confined to the Old World, *Protoceras* can have but a remote connection, but just when and how this connection was established cannot at present be determined. There are, however, certain American genera which are usually referred to the tragulines and which probably are more or less distantly related to that group. These genera are *Leptomeryx* and *Hypertragulus;* they are much alike, and yet with such significant differences as show that they are to be regarded as divergent branches of the same stock. One is tempted on zoögeographical grounds to assume a relationship to these genera on the part of *Protoceras*. *Leptomeryx* has an entirely different type of skull from that of *Protoceras* and one which closely resembles the structure of the traguline skull, differing merely in the concavity or flatness of the occiput, the small size of the auditory bullæ and their freedom from cancellous tissue, and in the presence of a fontanelle or vacuity between the frontal, lachrymal, and nasal. The odontoid process of the axis is conical. In the dentition, however, even in details, there is much likeness between this genus and *Protoceras*. In the character of the limbs *Leptomeryx* is in many respects in advance of *Protoceras;* thus, in the manus the magnum and trapezoid are ankylosed and the second metacarpal is excluded from the magnum. But the scapula, ulna and radius, metacarpals and phalanges, are very much alike in the two genera. Pelvis, femur, tibia, and fibula do not differ in any important respect, but *Leptomeryx* has a posterior cannon-bone and coössified cuboid and navicular. In spite of the many differences, there is an undeniable likeness of habit between *Leptomeryx* and *Protoceras*.

In *Hypertragulus* we find certain characteristics peculiar to itself, such as the retention by the lower canine of its original form and function, the loss of the first and isolation by diastemata of the second lower premolar, and the coössification of the ulna and radius, but the general resemblance to *Leptomeryx* is close. The structure of the cranium and position of the orbits are the same in both, but the elongated, constricted, and slender muzzle, the large and irregular fontanelles which encroach upon the nasals, the character of the palate, the shape and position of the posterior nares, the aspect of the base of the skull, with auditory bullæ and glenoid cavities, are all suggestively like what we find in *Protoceras*. The premolars have the same simple structure found in the latter, but are not so much elongated antero-posteriorly. The pes is like that of *Protoceras*, except for the coössification of cuboid and navicular. In size *Hypertragulus* somewhat exceeds *Leptomeryx*, but is much inferior to *Protoceras*. Still another genus of apparently this same group is the curious little *Hypisodus*, from the White River, with its hypsodont molars and ten functional lower incisors, made up of the incisors proper, the canines, and first premolars. The distal end of the fibula is coössified with the tibia, and the feet, so far as known, resemble those of *Leptomeryx*.

This family represents a group of White River selenodonts, each of whose genera has become more or less specialized in a way peculiar to itself, and with a tendency to simulate the Pecora in some respect or other, yet always retaining a number of primitive features. I cannot but believe that *Protoceras* represents a divergent offshoot of the same stock which, retaining in most respects the foot-structure belonging to the common ancestor of all these genera, has, at the same time, wonderfully paralleled the higher Pecora in many features of the skull. We have yet to find the forerunners of this genus in the two lower divisions of the White River formation, the *Oreodon* and *Titanotherium* beds, but one of these forerunners may prove to be the problematical genus *Stibarus*. The Uinta formation may be expected to yield the ancestor common to the entire group, and when it is found we shall prob-

ably discover in it the starting-point both of the Pecora and of the Tragulina. The manus of *Protoceras* appears to have been but little modified from that of this hypothetical form, and some such type of manus might easily give rise to those of all the later representatives of these various selenodont lines. The hypothetical genus will doubtless also be found in



the Old World, for nothing seems clearer than that both Pecora and Tragulina originated in that region, and the latter group, as narrowly defined, has always been restricted to it. From this supposed Uinta genus lead no less than four divergent lines in the White River. *Leptomeryx* has kept nearly the primitive type of skull, but has developed somewhat complex premolars, with some advance in the structure of the manus and still more in that of the pes. *Hypertragulus* has a somewhat modified skull, but very primitive dentition, with little change in the extremities beyond the coössification of the ulna and radius, and of the cuboid and navicular. *Hypis-*

odus has developed a hypsodont type of molar and curious lower incisors, while in *Protoceras* the principal modifications have been those of the skull.

Until the forerunners of *Protoceras* have been found, these conclusions can be only conjectural, but such a solution of the problem offers fewer difficulties than any other now available. The table on the preceding page displays the position of the genus according to this view.

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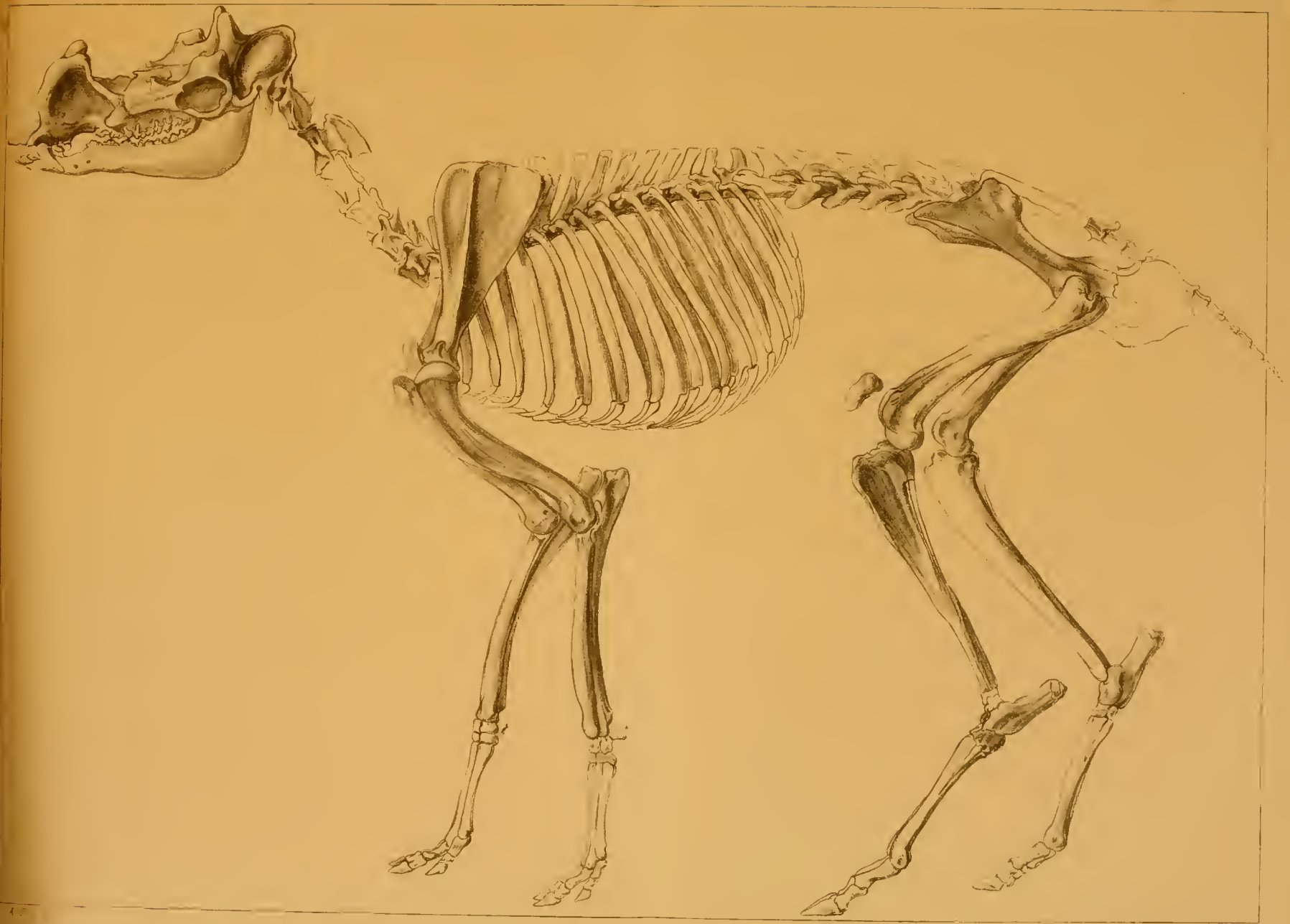
EXPLANATION OF PLATE XX.

Restoration of *Protoceras celer*, one-third natural size. The skull is taken from Osborn and Wortman's figure of the male, drawn to the proportionate length of the female skull which belongs to the skeleton. The secondary sexual characters being principally confined to the head, there is nothing misleading in this association of the sexes in one figure, especially in one of such small scale. The remainder of the skeleton belongs to a single individual, with the exception of the humerus, atlas, 12th thoracic and 1st caudal vertebræ, which are supplied from other specimens.



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EXPLANATION OF PLATE XXI.

(All the figures, except Nos. 5 and 12, are two-thirds natural size.)

FIG. 1. Skull of immature female, from the side. *Pa*, parietal; *Pa'*, parietal protuberance; *F*, frontal; *L*, lachrymal; *Na*, nasal; *P3*, third upper premolar; *D4*, fourth upper milk molar; *M1*, first upper molar.

FIG. 2. Skull of adult female, base-view. *Bs*, basisphenoid; *Ty*, tympanic; *C*, *P1*, alveoli of canine and first premolar.

FIG. 3. Skull of female, rear view; same specimen as Fig. 1. *Poc*, paroccipital process; *Pa'*, parietal protuberance.

FIG. 4. Inferior dentition, crown view. *C*, *P1*, alveoli of canine and first premolar; *M1*, first molar.

FIG. 5. Superior milk dentition, crown view. *D2*, *D3*, *D4*, second, third, and fourth milk molars; *M1*, first true molar. Natural size.

FIG. 6. Atlas, from above. *Sn*, foramen for first spinal nerve.

FIG. 7. Axis, side view.

FIG. 8. Sixth (?) thoracic vertebra, from the side.

FIG. 9. Third lumbar vertebra, from above. The centrum is concealed in the matrix. *met*, metapophysis; *tr*, transverse process; *pzy*, postzygapophysis.

FIG. 10. Scapula of right side.

FIG. 11. Right ulna and radius, seen from the external side. *R*, radius; *U*, ulna.

FIG. 12. Outline of distal end of ulna and radius. Natural size. *R*, radius; *U*, ulna; *S'*, *L'*, *C'*, facets for the scaphoid, lunar, and cuneiform.

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EXPLANATION OF PLATE XXII.

(All the figures, except Nos. 14, 19, and 20, are two-thirds natural size.)

FIG. 13. Right femur, front view.

FIG. 14. Right femur, proximal end. Natural size. *Tr 2*, second trochanter.

FIG. 15. Left patella, from behind. *In. Pr*, process for mesial face of femur.

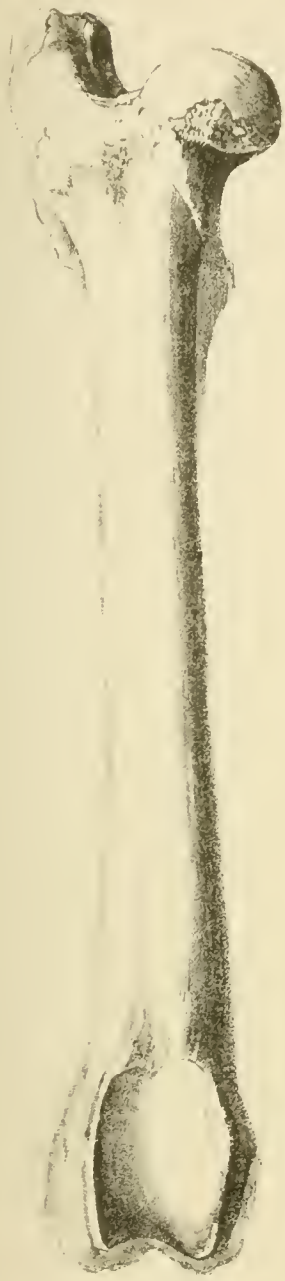
FIG. 16. Right tibia and fibula. *F*, fibula.

FIG. 17. Left manus, *II*, *III*, *IV*, *V*, second, third, fourth, and fifth digits. *S*, scaphoid; *L*, lunar; *Cn*, cuneiform; *Td*, trapezoid; *M*, magnum; *U*, unciform.

FIG. 18. Right pes, *III*, *IV*, third and fourth digits. The divergence of the distal ends of the metatarsals is not normal, but due to a distortion of the specimen.

FIG. 19. Phalanges of third digit of left manus, seen from the radial side. Natural size.

FIG. 20. Phalanges of third digit of left pes, seen from the tibial side. Natural size.



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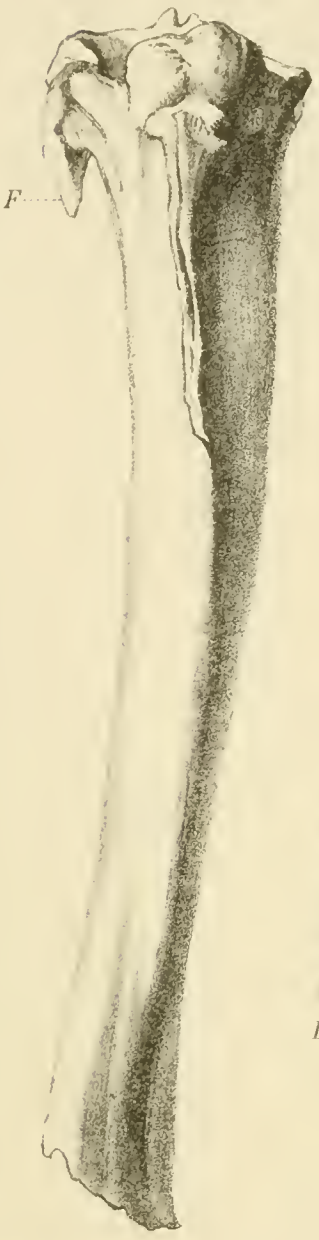

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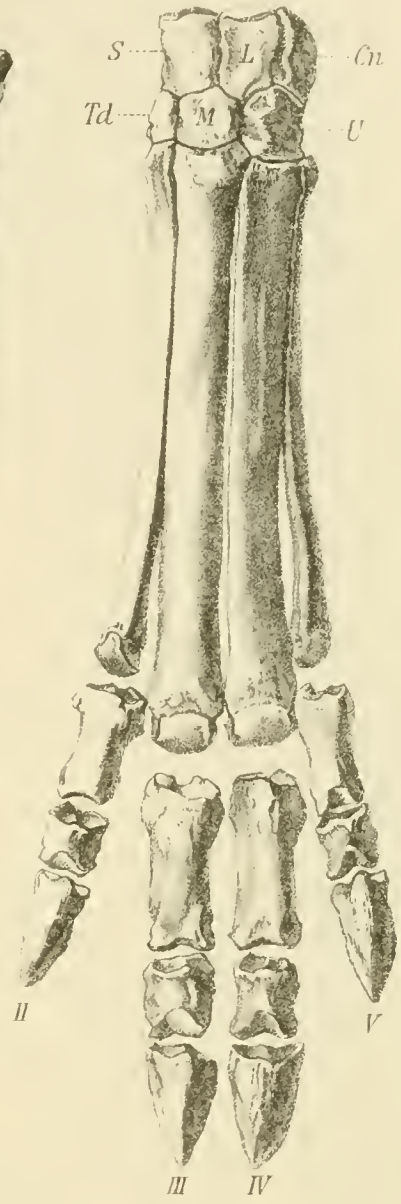

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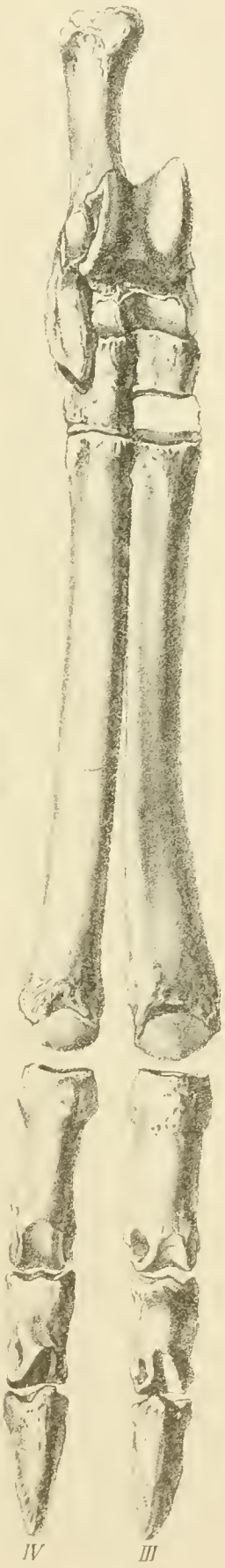

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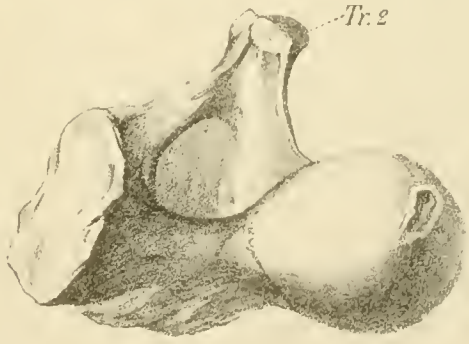

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A CONTRIBUTION TO THE ANATOMY AND PHYLOGENY OF *AMPHIUMA MEANS* (GARDNER).[1]

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THE anatomy of the adult skull of this eel-like Amphibian has been well described by Wiedersheim (1). Osborn (8) has given a most excellent description of the central nervous system. Hay (2) has given an interesting account of the finding of its eggs in an Arkansas swamp in September of 1887, and subsequently published the results of his study of the embryos contained in these eggs, and also of the skull of a small specimen six inches long. Cope has furnished a general description of the species, and endeavored to trace out its phylogeny by means of the insufficient data at hand. Kingsley (5), through a study of Hay's embryos, for the most part confirmed the latter's account, and also added to our sparse knowledge some important points, especially along the line of phylogeny. The writer has published a description of the conical arrangement of the muscles (6), and in a later article the manner of the fertilization of the eggs (7). No other investigations of any importance than the above mentioned have been made on this peculiar Amphibian, owing undoubtedly to the fact that the embryological material is so difficult to secure. The animals will not breed in captivity, and the batches of eggs laid are so few and so well concealed as to escape the sharp eye of the naturalists. Since the eggs are fertilized internally, a comparatively short period of external incubation is necessary, thereby limiting the probability of being discovered. Previous to February, 1894, no one had secured a specimen of the young less than six inches in length. In that month I was so

[1] I was very materially aided in making these investigations in the Princeton Biological Laboratory by Mr. C. W. F. McClure who placed at my disposal a large number of Amphibians and gave me numerous valuable suggestions.

fortunate as to have sent me, by a North Carolina dealer in embryological supplies, several very young specimens varying in size from seventy-eight to ninety millimetres in length.

In this paper it is my intention to give a detailed account of the anatomy of these very young specimens, and by comparisons with the adult structures as well as with other Amphibians to deduce a few new points in phylogeny. Since there has never been published any complete and reliable account of the anatomy of the adult, it will be necessary for me to begin at this point.

External Features of the Adult.

The general form is serpentine, having the same proportions as the body of a teleost eel. The circumference of the largest specimen I have seen was 150 mm., and its length almost one metre. The tail occupies about one fourth the length of the animal, and is laterally compressed in such a manner that the area of a cross-section is triangular with the apex at the dorsum. The body is slightly contracted just anterior to the fore limbs forming the so-called neck. The head is twice as long as it is broad, and vertically compressed. The snout is obtusely rounded, but is more pointed than in other urodeles, and extends from six to eight millimetres beyond the lower jaw. The lips of the upper jaw overhang for the most part those of the lower, thus preventing the mud from entering its mouth during its subterranean excursions. The eyes are one and a half millimetres in diameter, have no lids, utilize the epidermis as a cornea, and are situated along a transverse line cutting off the anterior third of the head. The anterior limbs are from fifteen to twenty millimetres in length, and support two or three diminutive digits. The posterior limbs, situated just anterior to the vent, are from fifteen to twenty-five millimetres long, and also have two or three digits. Cope (4) says that specimens have been found having two digits on the anterior limb and three on the posterior.

A few millimetres anterior to the fore limb is the branchial fissure, securely guarded by two membranous flaps. The skin is smooth, silky, and of a dark brown color dorsally, and of a

slate gray ventrally. The head is covered with mucous pores, arranged in several rows which unite in the region of the neck *so that only two distinct rows are seen on each lateral area of the body.* Cope (4) errs in saying none are present on the body.

Bones of the Head.

The head of *Amphiuma* is long and narrow, the general outline being somewhat like that of the skull of the Proteus except that the snout is not so pointed. While in the majority of Amphibia the skull is as broad as long, in *Amphiuma* it is twice as long as it is broad. It is composed of twenty-eight distinct bones : two maxillaries, a premaxillary, two nasals, two frontals, two prefontals, two orbitosphenoids, two vomero-palatines, two parietals, two exoccipitals, two proötics, two pterygoids, a parasphenoid, two stapes, two quadrates, and two squamosals. The maxillary is an irregular, oblong bone with a triangular cavity beneath, along one side of which are attached twenty-two conical teeth. This bone is not curved as in the majority of amphibians, but presents a straight alveolar edge. The foramina (Fig. 1, *c*, *b*) are seen on its dorsal surface, the anterior of which gives passage to small blood vessels and nerves. Cope (4) believes that the larger foramen is prophetic of the tentacular canal of the *Gymnophiona*. The maxillary articulates dorso-laterally with the prefrontal and nasal, ventro-internally with the vomero-palatine, and anteriorly with the premaxillary, which in *Amphiuma* is a single bone. This very irregular bone is composed of three parts : the alveolar portion (Fig. 11, P) bearing ten teeth, the dorsal wedge extending backwards between the nasals and frontals to a line joining the orbits, and a ventral wedge lying in the roof of the mouth between the vomero-palatines and parasphenoids. The greatest width of either of these wedges is one millimetre, and the length of each is about one third that of the skull. The nasals are small bones very much pitted, and serve to roof in the anterior part of the nasal chamber. The frontals are the longest bones of the skull, and bound the fore brain both dorsally and anteriorly. They extend underneath the posterior edge of the

dorsal wedge of the premaxillary, and the union of the descending plates at this point functions as a nasal septum, as seen in Fig. III. Through this portion of the bone is the canal for the passage of the olfactory nerve. Hay (2) does not believe that such a canal exists, though Wiedersheim (1) has correctly described it. Cope (4) has called the descending union of the frontals the ethmoid. In this he is wrong, as embryonic investigations (3) have clearly shown. I think the Sarasins (9) have followed Cope in this error, and together with him have sought to show relationships which do not exist. The frontals are in contact with the nasals and premaxillaries anteriorly, and laterally join the prefrontals and orbitosphenoids. Upon reaching the frontal bone the sagittal crest of the parietals divides, each fork running to the outer side of the former bone. In the groove thus formed lies the temporal muscle. The prefrontals take part in roofing the nasal chambers, have a rough surface and an irregularly oblong outline. They join the maxillaries laterally and form part of the orbit. The orbitosphenoids are small, taking part in the lateral boundaries of the brain cavity. They articulate with the parietals, parasphenoid, and vomero-palatines. They are almost separated from the frontals by the interposition of a narrow wedge of the parasphenoids. The vomero-palatines bear the inner concentric row of teeth, which number about forty-four. *The number of premaxillo-maxillary teeth is never less than fifty.* The number is wrongly stated by Cope as thirty-one. The vomero-palatines, together with the ventral wedge of the premaxillary, form the roof of the mouth. This pair of bones unite anteriorly, and are nowhere separated more than two millimetres from each other. Their backward extent ceases slightly posteriorly to the beginning of the parasphenoid. The parietals are the largest bones of the skull, and form the roof of the greater part of the brain cavity. Their median juncture is the sagittal crest. Their external borders are deflected upwards to form the temporal crests, thus giving rise to a broad groove for the reception of the powerful temporal and masseter muscles. The proötics and squamosals lie lateralwards of these bones, and posteriorly are joined by the exoccipitals which bear the two

pedestals for articulation with the axis. The exoccipitals join each other only in a very small part of their length, being separated by a V-shaped opening which is the foramen magnum. Immediately within this aperture, on either side, is seen a small facet for articulating with the prezygapophyses of the axis. The large foramina seen at the external sides of the bases of the pedestals give passage to the vagus and hypoglossal nerves. The exoccipitals are in apposition laterally with the squamosals and stapes. They do not reach the proötics, which lie anterior to the squamosals and external to the parietals, with which they join in such a manner as to form the anterior parts of the temporal crests.

The pterygoids are wing-like bones extending from the quadrates forward along the parasphenoid to near where the vomero-palatines arise, at which point the bone gives way to cartilage. The parasphenoid is the broad basal bone of the skull, extending throughout more than half its length. It is broadest in the otic region, and narrows in either direction. Its posterior part is bounded on three sides by the exoccipitals. Anteriorly, it extends beneath the ventral wedge of the premaxillary. The stapes is an orbicular bone, scarcely three millimetres in diameter, articulating with the parasphenoid, the exoccipital, and quadrate. It does not form a part of the suspensorium. The quadrate is a comparatively small bone, lying on the inner side of the descending squamosal, and joins the pterygoid, stapes, and parasphenoid. This bone, with the squamosal, forms the suspensory apparatus. It bears the facet for articulation with the mandible. The squamosal is another peculiar bone in the Amphiuma skull. *Whereas in most of the Urodeles it is directed forwards and slightly outwards, in Amphiuma it is directed outwards and downwards, and but very slightly forwards.* It is an exceedingly strong bone, and is firmly adherent to the exoccipitals, parietal, and proötic above, and joins the quadrate and stapes beneath. The possession of this bone, according to Cope (4), allies the families *Amphiumidae* and *Coecilidae*. The bone which Cope has called squamosal in the Coecilians is quite differently located, being directed forwards and inwards in such a manner as to form

part of the orbit, and therefore deserves the name quadratojugal, as some authors have already called it.

The mandible is a simple structure, each ramus being composed of these bones, *viz.*, the dentary, the angular, and articulatory. The dentary supports twenty-two teeth, and forms the whole external portion of the ramus, and appears for some distance in front on the inner side. The external surface presents a foramen for the mandibular nerve. The angular and articular are so well coössified for the most part that their boundaries cannot be clearly defined. Anterior to the facet for articulation with the quadrate, the articular abruptly rises into a prominence, to which the temporal muscle is attached. The angular lies beneath the articular, and forms a projection behind it, in which the digastric muscle is inserted. Immediately anterior to the condyle is a notch for the insertion of the masseter muscle. The rami are not anchylosed in front, but are held together by cartilage.

The hyoid apparatus of *Amphiuma* is quite unlike that of any other Amphibian (Fig. XV). There is but one basibranchial to which are joined two ceratobranchials bound by cartilage. There are four epibranchials. The basihyal is cartilaginous, as are also all the epibranchials except the first. The hypohyals are very short, being scarcely more than one-half as long as the basibranchial. The ceratohyals are longer than the ceratobranchials. The few cartilaginous formations of the skull not already described will be discussed later.

The Limbs.

The limb bones of *Amphiuma* are characteristic of reptilia in some respects. The fore limb consists of a humerus, ulna, and radius, carpus, metacarpus, and phalanges. These bones are proportioned with reference to each other, as in mammalia. The humerus of a specimen one metre long is about one centimetre long. Its head is of cartilage. Immediately below the head, on the anterior side, is a prominence for the insertion of the biceps muscle. The distal third of the shaft is slightly flattened to afford a more advantageous surface for the articu-

lation of the two bones of the forearm. These bones are approximately of the same length, but the radius is the stronger. The carpals are not ossified. They are five in number. The ulna articulates with the ulnare only, but the radius articulates with both the ulnare and radiale. There may be either two or three metacarpals. Formerly this difference in number served as basis for the specie classification, didactyla and tridactyla. Professor Ryder has since demonstrated the identity of the two forms. The second and third digits have two phalanges each. The fourth digit has only one.

The hind limb of *Amphiuma* is fully one-third longer than the fore one. The femur has a well developed, cartilaginous head and a prominent trochanter. It broadens gradually from the middle to the distal end. The tibia and fibula are a trifle over one-half the length of the femur, and are approximately equal to each other in strength. The tibia is largest at its proximal end, but the two ends of the fibula have equal surfaces. These bones articulate with the tibiale and fibulare of the tarsals. The third tarsal supports the third and fourth metatarsals, and the tibiale supports the second metatarsal. The second and third metatarsals have each two phalanges, but the fourth has only one. All the phalanges and metatarsals are well ossified, but the tarsals are cartilaginous. The girdle bones are less perfectly formed than the limb bones. The shoulder girdle consists of a cartilaginous coracoid, a bony scapula, and a cartilaginous suprascapula. There is no evidence of true sternal elements. The pelvic girdle is more complete, having an ischium, ilium, and pubes. The ischium and pubes are united to each other and also to their fellows of the opposite sides, so as to form a shield-like plate, which is composed of cartilage, with the exception of two discoid ossifications in the posterior parts. The acetabulum is entirely cartilaginous. The ilium proper is well ossified, very slender, and surmounted by a cartilaginous style which is attached to the sacral rib of the sixty-third vertebrae on the right side and the sixty-fourth on the left. I think Mr. F. A. Lucas of Washington was the first one to observe this asymmetrical disposition of the iliac bones.

The Vertebrae.

Three divisions of the vertebrae may be recognized: cervical, trunk, and caudal. There is only one bone in the first division known as the axis, the atlas being anchylosed with the skull. The anterior face of the axis presents two concavities for articulation with the occipital condyles. There are also two slight projections between the concavities, which may be called prezygapophyses, as they are applied to the internal facets of the condyles. On the posterior aspect are seen the postzygapophyses descending from the backward extension of the neural arch. The neural spine is only slightly developed, and there are no transverse processes. This vertebra as well as all the others is amphicoelous. The trunk vertebrae number sixty-two. All have prominent transverse processes and high neural spines. The transverse processes of the first seven or eight vertebrae are laterally sulcated in their distal regions, and have short ribs attached. The neural spines bifurcate posteriorly and send their prongs outward on the postzygapophyses. The course of each prong is V-shaped, with the apex directed anteriorly. From this apex a small diapophysial spine extends forward to near the anterior base of the neural spine. This process serves a special purpose in Amphiuma, as I shall show later. The faces of the zygapophyses are in a symmetrical plane, extending in an axial direction. All the trunk vertebrae except the first two have small hypapophyses attached to the anterior part of the body, which project anteriorly. The middle two-thirds of the body of each vertebra is so constricted laterally as to form a rather sharp spine viscerally.

There are two sacral vertebrae. Their processes are the same as the trunk vertebrae. The caudal vertebrae number thirty-seven, making a total of one hundred and one bones in the spinal column. All the caudal elements except the first two have prominent chevron bones. The neural spine, which is so high in the trunk region, is very much depressed, and the posterior bifurcations of this spine are more extensive. The transverse processes are declivous and decrease in length posteriorly as far as the mid-tail region, where they entirely dis-

appear. The parapophysial spine remains constant for the important muscular attachments. The many processes and depressions characterizing the bones of *Amphiuma* present but slight genealogical significance until we have made a careful study of the muscular system.

Muscular System.

During the past six months I have searched carefully for a description, or even a few words of introduction, to the muscular system of this strange animal, but have been able to find only a very terse discussion of the subject. This is given by Dr. Bronn (10), and consists of a few words concerning the muscles of the head. A brief account of the dorsal muscles was published by the writer (6) in April, 1894. A satisfactory dissection of the muscles requires considerable and careful preparation of the tissues, owing to the fact that the muscular arrangement is so complex and many of the muscles are so minute and massed together. After much experimenting I found the following fluid a most admirable agent for the maceration and differentiation of the muscular elements: one part of one-fourth per cent chromic acid, two parts of ten per cent nitric acid, two parts of seventy per cent alcohol, and three parts of water. The specimen may be left in this fluid a week, at the end of which time it must be thoroughly washed in running water for several hours. Then the muscle-fibres will be found stained a bright red, while the fascial envelopes will remain uncolored and the tendinous origins and insertions will be swollen so as to be readily seen.

Great difficulty is experienced in neatly separating the skin from the underlying muscles, since the two are indissolubly connected by an exceedingly tough fascia. This fascia consists of a dense sheath of tissue arising from the neural spines in two plates, which, scarcely separated at their origin, diverge gradually as they rise to the dorsal surface, thereby bounding laterally an area whose cross-section is triangular. This area is filled with a loose connective tissue and fatty substance. Each plate of fascia is reflected over the external surface of

its respective side. *At a distance of one-fifth of the circumference from the dorsal line, a cleavage into two membranes takes place, one of which descends almost vertically through the body-wall to the cavity where it gives rise to the transversalis abdominis muscle.* This muscle continues ventralward until within about one centimetre of the mid-ventral line, where it becomes fascia. The other reflected plate of fascia extends subcutaneously around the body to the mid-ventral line, where it comes in contact with the internal plate, since no muscle takes part in the formation of the body-wall in the mid-ventral region. This tough fascial sheath also envelopes the head, being strongly attached to the median keel in the posterior region, and broadly adhering to the anterior portion of the frontal, the prefrontal, nasal, and premaxillary bones.

Muscles of the Head.

Amphiuma has four dorsal head-muscles : pterygo-maxillaris, masseter, temporal, and cervico-parietalis. The pterygo-maxillaris arises mainly from the median juncture of the parietals and the fascia covering the horizontal surface of the frontal. A small portion of the muscle is a continuation of the cervico-parietalis. Its insertion is on the dorsal side of the pterygoid bone and cartilage. It is clear to be seen that this muscle in *Amphiuma's* ancestors must have been the anterior part of the cervico-parietalis. The tendons of the temporal, through enlargement and continual activity have usurped almost the entire space of the parietal groove, thereby causing the unused muscle to dwindle. The masseter is an exceedingly strong muscle, and arises in two parts. One part originates from the lateral area of the proötic, the other from the anterior curved keel of this bone. The two unite almost at their origin, and extend as a thick, muscular mass to its insertion on the mandible external to the coronoid process. The temporal is the long and strong elevator of the lower jaw. It arises from the neural spines of the fifth, fourth, third, second, and first vertebrae. It is inseparably joined with its fellow as far as the parietal bone. At a distance forward of this equal to the interorbital space, the

muscle is transformed into two tendons which, passing along the parietal groove, descend anterior to the proötic, and are inserted together in the coronoid process. The arrangement of this muscle is such as to give great strength and yet preserve the flat attenuate condition of the head in the proötic region. The cervico-parietalis muscle arises from the second and first vertebrae, and is attached to the posterior part of the parietal and the dorsal area of the exoccipital bone. The lateral head-region presents five muscles: cucularis, digastricus-maxillae, interbranchiales constrictores arcuum branchiarum, levatores arcuum, and adductores arcuum. The cucularis arises from the fascia of the transverse processes and descends a narrow band of muscle anterior to the forelimb to its insertion in the walls of the oesophagus. The digastricus maxillae is a large flat muscle arising in three portions. The first portion is attached to the neural spines in the shoulder region and is a continuation of the superior dorsal muscle. The second portion arises from the summit of the first epibranchial and mingles inseparably with the first. The third portion is the strongest, and arises from the posterior otic region, joining the other two immediately, whence the entire mass passes downwards and forwards to a firm insertion in the posterior angle of the lower jaw. Bronn's Their-Reichs (10) describes only two portions as origins of this muscle. The writer has detected several errors in this work in the descriptions of the muscles of the head of *Amphiuma*. The interbranchialis constrictores arcuum branchiarum exist as thin oblique bands of muscular fibre between the first, second, and third epibranchials, but no fibre joins the third and fourth, between which the gill slit persists in the adult. The levatores arcuum arises from the inferior side of the posterior portion of the digastricus and descends as a flat band of fibres to its insertion on the summits of the epibranchials. The adductores arcuum consist of a tendinous band connecting the summits of the four epibranchials, whence it extends downwards and backwards to a point above the forelimb, where its course becomes transverse, forming the third inscriptio tendinea. The ventral head-region presents eight

muscles : thoracico-hyoideus, omo-humero-maxillaris, genio-hyoideus, mylo-hyoideus, stylo-hyoideus, genio-glossus, cerato-hyoideus externus, and trachealis arcuum. The thoracico-hyoideus is a large muscle extending from the median extremity of the cerato-branchial backwards until it is inseparably mingled with the rectus abdominis. Its fibres are interrupted by several inscriptiones tendineae, which are present as far forward as the gill slit. The omo-humero-maxillaris is well developed, arising from the fascia ventralward of the fore limb, and increasing in strength as it runs forward to its insertion on the angle of the maxillary. The genio-hyoideus is a thin band of muscle, arising from the symphysial region of the mandible, and is inserted in the fascial sheath of the thoracico-hyoideus. The mylo-hyoideus forms a thin sheet of muscular fibre extending transversely between the rami. The stylo-hyoideus lies posterior to and deeper than the former muscle, and extends from the cerato-branchial to the cerato-hyal and hypohyal bones and basi-hyal cartilage. The genio-glossus lies in the floor of the mouth parallel with the ramus connecting it with the hypohyal. The cerato-hyoideus-externus lies immediately beneath the stylo-hyoideus. The trachealis arcuum is composed of a transverse band of fibres extending from the fascia of the tracheal region to the tendon joining the summits of the epibranchials. Its function I believe to have been the retraction of these arches. Fischer, Duges, Humphry, Schmidt, Goddard, and Van der Hoeven disagree to such an extent upon the names of the muscles of the Amphibian head that I have not adopted any one man's nomenclature, but have retained the name which seemed most proper for the muscles of *Amphiuma*.

Muscles of the Limbs.

So far as I have been able to learn no one has yet attempted to describe the muscles of *Amphiuma's* limbs. The minuteness and massing together of the muscles render it a most difficult undertaking. On the ventral aspect of the fore limb are seen four muscles. The largest one, representing the

pectoralis major, arises from the fibres of the omo-humero-maxillaris posterior to the limb, and extending distalward as a radiate muscle, is inserted in the fascia of the muscles of the arm. Immediately beneath this muscle, which covers the entire coracoidal region, is found the supracoracoideus, a radiate muscle arising from the ventral surface of the coracoidal cartilage and extending to its insertion in the head of the humerus. Its function is that of depressing the fore limb. The slender fascia-like deltoideus arises insensibly from the fibres of the omo-humero-maxillaris, and runs along the anterior side of the arm, being slightly inserted on the distal end of the humerus, but continuing as a flexor carpi radialiis to its final insertion in the carpal cartilages. The coraco-humeralis is a mere branch of the omo-humero-maxillaris, and is strongly inserted in the anterior proximal region of the humerus. It draws the limb cephalad. The flexor digitorum communis is a greatly degenerated muscle arising from the middle part of the humerus and extending downwards to the carpal region. The dorsal aspect of the fore limb presents four muscles very closely bound together by dense fascia. A slender muscle representing the triceps brachii arises from the fascia posterior to the branchial arches, and appears to be attached slightly along its entire course down the arm to the phalanges. From the obliquus externus a band of fibres runs forward to its insertion in the upper part of the humerus, serving to draw the arm backward. This muscle corresponds to the latissimus dorsi. Another muscle arising in common with the last mentioned is inserted along the middle portion of the humerus, sending fibres onward to the phalanges, and is probably the atrophied remains of the infraspinatus. Owing to the fact that *Amphiuma* seldom bends its arm at the elbow, the muscles arising from the shoulder region in many instances continue to the forearm and hand. This is the primitive condition of limb muscles. *In fact, I do not think this animal is capable of flexing the forearm or the arm, as the muscles are so bound together by dense fascia and continuous at the elbow joint. My anatomical inference on this point was confirmed by observing a large specimen moving across the floor. The limbs did not touch the floor, but*

they were moved quite vigorously backward and forward, and were not bent at the elbow or knee joints.

The muscles of the hind limbs are larger and more distinct than the foregoing. On the ventral aspect are seen three muscles. The large muscular mass arising from the ischio-pubic symphysis and taking its course down the posterior side of the limb to the phalanges appears in the reptilia as the adductor and gracilis muscles. Immediately beneath this mass a radiate muscle arises from the ischio-pubic plate, and is strongly inserted in the greater trochanter. The femoro-caudal arises from the third and fourth caudal vertebrae, and descends forward in two parts, one of which is inserted in the upper part of the femur; the other joins with the semimembranosus extending down the posterior side of the leg to the insertion into the phalanges. The ischio-caudal is a well developed muscle originating on the posterior margin of the ischium and extending posteriorly to an insertion on the vertebrae of the anterior third of the tail. The pubo-tibialis is a strong adductor arising from the coelomic aspect of the ischio-pubic plate and extending across the middle part of the femur down the front side of the tibia to an insertion in the phalanges.

The dorsal aspect of the hind limb presents two muscles. The rectus femoris is a heavy muscle arising from the fascia in the region of the ilium and extending to the distal part of the femur, where it is attached, thence continuing to the aponeurosis of the foot. The ilio-peroneal arises from the ilium, and extends to the distal bones of the leg. Thus it will be seen that many of the muscles of this limb pass over two joints, thereby indicating very restricted movements, if any, in the knee joint. The phylogenetic significance of these facts will be discussed later.

Muscles of the Trunk.

The muscles of this region furnish a most intricate as well as a most interesting study. This portion of *Amphiuma's* muscular system had not been described prior to my paper in the *Anatomischer Anzeiger* of April, 1894. As was stated in

the early part of this communication, the trunk muscles are separated into two regions, *viz.*, the dorsal and abdominal, by the fascial lamina split off from the external dorsal sheath. This lamina extends through the wall to the body cavity. A similar disposition of the fascia occurs in *Cryptobranchus japonicus*, van der Hoeven, as described by Humphrey (11). The dorsal mass of *Amphiuma* is not differentiated into separate muscles, but for the sake of convenience may be considered as composed of two parts: the superior, lying above the transverse processes, and the inferior, lying beneath these processes. The former corresponds to the erector spinae of some authors. The latter is called rectus trunci internus by Schmidt, Goddard and van der Hoeven in a description of other Amphibia of this order. Mivart (12) speaks of a similar muscle in *Menopoma* as being a part of the retrahens costarum. The anterior portion of this muscle in *Amphiuma* undoubtedly functions as a retrahens costarum, being attached to the minute ribs found on the first seven or eight vertebrae of the trunk. The skin having been carefully removed from the back, and the muscles well stained and macerated by the fluid mentioned previously, there will be seen lying along the axis longitudinally-disposed rows of cones, the enveloping fascia of which appears, at first sight to form a kind of network.

In the superior dorsal mass there are three rows of cones lying side by side. The apices of the row adjacent to the axis are directed posteriorly, those of the next row anteriorly, and those of the third row posteriorly. *Thus it is seen that the apical direction of the cones varies alternately in the different rows.* Each cone is introduced into the preceding one about one-third of its length, as shown in Fig. 10.

From the exterior apex of each cone in the two distal rows a tendinous cord extends to the interior apex of the following cone, thus serving to hold the apices in position. The row most distant from the axis has the deep part of the base of each cone firmly attached to the outer half of a transverse process. That part of the base distal from the axis is reflected to form an inscriptio tendinea extending transversely to the mid-ventral line. The superficial base of the cone blends with

the fascial body investment. That side of the base proximal to the axis is continued forward as the distal side of a cone in the adjacent row. Therefore it is seen that a transverse line through the apex of a cone in one row will pass through the base of a cone in the adjacent row.

In the middle row the deep sides of the bases are attached to the post-zygapophyses and their spines. The distal and proximal sides of the bases are continued as the lateral boundaries of cones in the adjacent rows. The superficial sides of the bases have the same insertions as those in the row previously described. The cones in the row adjacent to the axis are somewhat flattened laterally by their close apposition to the neural spines. The deep side of the base of each cone is securely inserted on the postero-lateral division of the neural spine. The distal side of each base takes the same course as the corresponding side in the adjacent row. The proximal sides are fastened to the neural spine and also to the fascia arising from the neural spines to serve as the body investment. The superficial sides of the bases and also one-half of the superficial lateral boundaries of the cones are blended with the external fascial envelope. The apices of the cones in this row give off ribbon-like tendons which extend to the interior of the following apices. Such is the general arrangement of the cones in the superior dorsal mass.

The size of these cones varies. Those of the distal row are all of the same size, and are somewhat larger than those of the other two rows, the length being fully three centimetres, and the diameter of a base about one and a half centimetres. The length of a cone in the proximal row is scarcely two centimetres, and its base is about one-half a centimetre. The preceding measurements were made on an animal almost one metre long.

Since the arrangement of these cones is so regular, it is easy to estimate their number, which I have calculated to be three hundred and seventy-two in the superior dorsal mass.

A view of the inferior mass from within the body cavity reveals no evidence of a conical arrangement, but instead are seen, very prominently marked, the transverse septa at regular

intervals, corresponding to the lengths of the vertebrae. It will be noticed, however, that the septa appear to cease very abruptly at a distance of two-thirds of a centimetre from the axis. A careful dissection of a well stained specimen along this line brought to view the same conical arrangement observed in the superior mass. The cones in the distal and middle rows are quite perfectly developed, but those of the proximal row are very imperfectly formed, being too closely apposed to the spinal axis. The direction of the apices in these rows is exactly opposite to those in the superior mass; that is, the proximal row of cones has its bases pointing anteriorly, whereas in the corresponding row of the superior mass the apices pointed posteriorly. The cones are much smaller, being scarcely half as large as the overlying ones. The superficial sides of the bases, as well as a large part of the superficial lateral area, are inseparably united to the dense fascia lining the body cavity. The outer sides of the bases in the distal row are reflected to form the transverse septa, while the deeper sides of the bases are firmly attached to the lower side of the outer half of the transverse processes. The inner sides of these bases are continued to form the lateral boundary of a cone in the adjacent row. The attachments of the middle row are so similar to those of the same row in the superior mass that I will not give them. The apices of these two rows are connected with the interior part of the apices of the cones following by a ribbon-like tendon.

In the row adjacent to the spinal axis the deep sides of the bases adhere to the hypapophyses of one vertebra, and the apices are inserted on the hypapophyses of the vertebra following, so that each hypapophysis serves for the attachment of an apex and the deep side of a base. From this brief description it can be readily seen that the general plan of the cones is the same in both dorsal masses.

The conical arrangement of the muscles prevails not only in the dorsal portion of the tail of *Amphiuma*, but also in the ventral portion. The disposition and attachments of the cones here are so very similar to those of the trunk region that it would be unprofitable to describe them. The number of cones

in this region is approximately four hundred and sixty-eight, though some of them near the extremity are rather imperfectly formed. The whole number in the trunk, counting twelve to each vertebra, equals seven hundred and forty-four, which, added to the four hundred and sixty-eight in the caudal region, gives a total of one thousand two hundred and twelve cones. *In other words, the dorsal and caudal muscles of this animal have over one thousand strong fascial attachments.*

Having discussed the general structure of these muscles, it is now in order to determine the direction of the fibres. These are not parallel to a line through the apices of the cones, as we should expect, but are so directed as to form an angle of about ten degrees with that line. Since there are no cones found in the outer half of the dorsal muscle, the direction of the fibres there is exactly parallel with the axis, being, however, completely interrupted by the inscriptiones tendineae.

So far as I have been informed, this peculiar conical disposition of the fibres of the dorsal muscle has been observed in only two other vertebrates and in no other Amphibians. Dr. Hair (13) describes similar cones in the alligator, and I have noticed the same structure prevalent in *Sphoenodon* (*Hatteria*), the peculiar New Zealand lizard. The genealogical significance of this muscular arrangement will be discussed later.

The ventral trunk muscles are, as in other Urodeles, composed of four sheets of fibres: the transversalis abdominalis, the obliquus internus, obliquus externus, and rectus abdominis. The first named is a mere continuation of the descending lamina of the external dorsal fascia. It may therefore be said to arise from the neural spines, and with its fellow, form a tube inclosing the viscera and dorsal muscles. In other words, the lamina may be considered as an aponeurosis, the muscle-fibres originating just before the aponeurosis emerges into the body cavity. The muscle passes transversely around the ventrum, dwindling again to fascia about one centimetre before joining its fellow along the mid-line. *The striking feature in this muscle is that it is unaffected by the inscriptiones tendineae, a condition not present in any other Amphibian. The obliquus internus and also the obliquus externus are thicker on the left*

side of Amphiuma than on the right. The left dorsal mass is also considerably stronger than the right. This asymmetry is probably due to the manner in which the animal lies coiled, though I have not had opportunity to demonstrate that fact. The obliquus internus muscle is composed of fibres taking origin from the tendons of the transverse processes, and extending obliquely anteriorly between the inscriptiones tendineae. External to this muscular plate lies the obliquus externus, readily recognized, as the fibres extend obliquely posteriorly between the inscriptiones. As the fibres of these two oblique muscles approach the ventral line they gradually change their direction, becoming finally parallel with the axis of the animal, and thus form the rectus abdominis. In all other Amphibians except *Amphiuma* this muscle is continuous over the ventral line. Its fibres are completely interrupted by the inscriptiones. Anteriorly it is continuous with the thoracico-hyoideus and omo-humero-maxillaris. Posteriorly it is attached to the pelvic elements, but continues as the ventral caudal muscle. Thus it will be noticed that the trunk and head muscles of Amphiuma are more highly specialized than those of other Urodeles, while the limb muscles are less specialized.

Digestive System.

The food of this Urodele consists of crayfish, small teleosts, and other similar aquatic life. The lining membrane of the buccal cavity is tough and smooth, but its continuation into the pharyngeal and oesophageal region is loose and somewhat corrugated longitudinally. On the ventral side are numerous ciliated columnar cells. The stomach is a slight enlargement of the oesophagus, beginning at a distance behind the shoulder girdle, equal to the distance of that girdle from the tip of the nose. The mucous lining of the stomach is thrown into small longitudinal ridges in the anterior portion of the organ, these ridges increasing in prominence as they extend posteriorly. The walls of the stomach in its anterior parts are but little thicker than those of the oesophagus, but posteriorly they become nearly twice as heavy. The length of the stomach is

equal to almost one-third of the distance between the fore and hind limbs. The transition from stomach to duodenum is readily recognized by the vast difference in the thickness of the wall, that of the latter being very thin. The membranous vascular folds increase in prominence and continue throughout the entire intestine to the rectum. The intestine is for one or two centimetres folded upon itself at two or three points in its course. The pancreatic gland lies dorsalward of the posterior half of the stomach, and for an equal distance along the duodenum. The liver extends from the region of the tenth to the thirty-eighth vertebra, being almost twice as long as the stomach. It is entire. The gall bladder lies near the caudal termination of the liver. The rectum consists of an abrupt expansion of the digestive canal at a distance of four or five centimetres from the cloaca. The internal wall of the rectum is quite smooth. This portion of the canal passes gradually into the cloaca, recognized only by its location and smaller diameter. The cloacal region will be described in the discussion of the urogenital apparatus.

The Circulatory System.

This system in *Amphiuma* is very much the same as in the salamanders. The heart is surrounded by a very large sac, through which may be seen the ductus cuvieri entering the large auricle. The bulbus arteriosus is long, giving off an aorta bow on each side. The carotids are exceedingly large. The iliac arteries and veins are very prominent. The venae revehentes are clearly visible in the kidneys. The portal vein lies along the dorsal side of the liver, receiving its numerous tributaries from the intestines, pancreas, and liver. The pulmonary vein is distinctly visible, passing to the apex of the lung on the external wall. Other features of the vascular system are in common with the order of Urodeles.

I shall not attempt a description of the nervous system, as that has been admirably discussed by Dr. Osborn (8).

The Genito-urinary System.

The kidneys are leguminoid bodies located immediately anterior to the hind limbs. In my largest specimens they measured four centimetres in length. The ureter is very short. The urinary bladder is exceedingly elongated, extending from the termination of the liver to the cloaca. *Cope has asserted that Amphiuma has only one testis, but I find paired testes extending from the liver half way to the vent.* In alcoholic specimens they are of a brownish spongy texture. They are attached to the body wall by folds of the peritoneum. A mesonephric or Wolffian duct is present as a thick-walled tube running in a straight course from a point near the gall bladder to the urogenital sinus. The vesicula seminalis is a thin-walled tube ending blindly in front near the gall bladder and extending in a convoluted course to a common opening with the mesonephric duct into the urino-genital sinus. The vasa efferentia are present as delicate tubes arising from the testis and emptying into the Wolffian duct, which acts as a vas deferens. The ovaries are very slender bodies lying a short distance posterior to the liver. The oviducts extend along the dorsal side of the body wall, terminating in the urogenital sinus. It has for a long time been a question whether the eggs of *Amphiuma* are fertilized internally or externally. I published a short article relating to this subject last April (7). I am now fully convinced that internal fertilization takes place.

In the early part of the spring of 1893 I secured a male specimen of *Amphiuma* a trifle over a metre in length in Northern Tennessee. This animal was the largest of its kind on record, and was found farther north than any previously discovered. On examining the vent I found exuding a viscid substance which, when placed under the microscope, revealed numerous spermatozoa. The inner walls of the vent were covered with dense papillae on their posterior parts. These papillae under the microscope proved to be the orifices of numerous glands which secreted the almost colorless waxy substance in which the spermatozoa were lodged. *The anterior parts of the internal vent walls are furnished with from fifteen*

to twenty membranous laminae extending obliquely from within outwards and backwards in such a manner as to transfer the generative products slowly from the cloaca to the external lips of the vent. When these lips are placed in apposition to the lips of the female vent, the reproductive agents are induced within the cloaca of the latter by means of a series of capillary tubes (Fig. XI, *C*) *arranged on the inner walls of the vent and extending from without inwards and forwards.* I do not see how these different features in the vent structure of the two sexes can serve any other purpose than that which I have described. Furthermore, the fact that the male was so filled with spermatozoa as to cause them to exude indicates that that month, May, was the natural time for their evacuation; and, inasmuch as the eggs are not deposited until August or September, fertilization must occur within the body of the parent. This theory of internal fertilization is further strengthened by the fact that *Ichthyophis glutinosus*, the blind-worm of Ceylon, an animal closely related to the family Amphiumidae, is reported by the Sarasins (9) to have its eggs fertilized internally.

The Respiratory System.

The anatomy of the respiratory apparatus in this animal is very simple. The external apertures of the nostrils are exceedingly small, being situated near together on the forward aspect of the fascial region. The internal nares appear lateralwards of the posterior limit of the vomero-palatine series of teeth. *The trachea is very long, being in my largest specimen nearly six centimetres.* The glottis is a small longitudinal slit on the ventral side of the pharyngeal region. There is no epiglottis. The trachea is thin-walled, without any cartilaginous formations. The lungs are annexed to the trachea dorsalward of the heart. Cope has greatly erred in saying the lungs are subequal. *The left lung is coextensive posteriorly with the liver, but the right one extends within three or four centimetres of the vent.* The diameter of the left is much smaller than the right also. The walls of these respiratory sacs are quite thick anteriorly, and grow thinner as they pass caudalward.

The transverse trabeculae in the cardiac region are ten millimetres apart, and are thrice as heavy as the longitudinal trabeculae. Immediately anterior to the front limbs is seen a spiracle guarded by membranous flaps to exclude the mud. This aperture is in communication with the pharynx, and is supplied with special muscles for closing it, as previously described.

Having now called attention to the important features of the adult, it is in order to make a brief examination of the young.

The Young Amphiuma.

For a long time embryologists have been seeking a good supply of specimens representing the development of this peculiar Amphibian. Hay (2) was successful in finding the eggs of *Amphiuma* in an Arkansas swamp in 1887. This material furnished important information. Last February I was so fortunate as to secure a number of very young specimens from a North Carolina dealer. They ranged in length from sixty-eight to ninety millimetres. They were found in a damp locality under some large rocks. Hay's embryos were 45 mm. long. It is probable that my specimens were hatched in November or December. The general shape of the young (Fig. 12) is very much the same as the adult. There are no signs of gills, and only one gill-opening persists on each side. The eyes are rendered useless by the heavy shields of epidermis, as in the adult. *The dermal glands are more prominent than in the old.* The caudal fin is less atrophied. The lower jaw is correspondingly shorter than the adult's. The projecting dermal folds of the upper jaw are little developed. *The legs are relatively longer than in the adult, but the digits are imperfectly formed, those on the hind limb being quite distinct, while there are no signs of any on the fore limb.* Hay (3) states the reverse of this to be true in his embryos, *viz.*, that the digits of the fore limbs are better differentiated than those of the hind. A gross dissection of this small specimen showed that the internal structure accorded with that of the adult, except in the case of the left lung, which did not extend to the caudal

termination of the liver. The most important information was gained from the study of the heads of several specimens stained *in toto* in borax-carmine and cut serially into sections one-fiftieth of a millimetre in thickness. Horizontal as well as sagittal and transverse sections were made.

The Skeletal Anatomy of the Head.

The skeleton of the head is shorter and broader than in the adult. The ossifications are, of course, vastly different. There are but sixteen fully ossified elements in the cranium: premaxillary, nasals, frontals, prefrontals, parietals, squamosals, maxillaries, vomero-palatines, and parasphenoid. The following elements are partially ossified: orbitosphenoids, proötics, and exoccipitals. The pterygoids, quadrate and stapes are wholly cartilaginous. The premaxillary is quite the same as in the adult, and needs no further description than to state that the interosseus septum is not derived from the nasal roofing cartilage, as Wiedersheim (1) has suggested. The two elements, though in contact, present no transition from one to the other. The enveloping cartilage of the nasal sac is incomplete where the osseous septum is present. The union of the cartilaginous floors of the nasal sacs medially extends beneath the anterior part of the brain for a short distance, when the middle portion of the cartilage passes into connective tissue, leaving two lateral bars. Hay (3) speaks of an unpaired piece of cartilage lying in the roof of the mouth of the adult between the anterior ends of the vomero-palatines, and states that it is not found in his embryos. Wiedersheim speaks concerning the same as follows: "Da wo die Vorderenden der Vomero palatine in der Mittellinie zusammenstossen, ragt ein conisch gestalteter Knorpelzapfen vom Boden der Nasenhöhle in die Schleimhaut des Mundes herab, von welcher er einen Ueberzug erhält."

Hay believes that this nodule of cartilage has been cut off from that forming the floor of the nasal sacs by the union of the bones in the roof of the mouth. Fortunately my specimen is of just the proper stage to settle the question. *The roofing*

bones of the mouth are fully ossified before the nodule is formed. In a specimen eighty-eight millimetres long the cells of the dense connective tissue are concentrically arranged preparatory to the formation of the true cartilage, which in a specimen ninety millimetres long has made its appearance. The frontal bone, though completely ossified, differs from the adult in the manner of giving exit to the olfactory nerve. Hay implies that Wiedersheim has erred in the following description: "Es handelt sich nämlich, wie am besten aus der Figur 20 F ersichtlich ist, um eine an der Unterfläche der vorderen Stirnbeingegend auftretende Knochenzwinge, deren mediale Circumferenz vorn und einwärts, und deren laterale mehr nach hinten auswärts gelagert ist. Beide stehen parallel zum Medianebeine und sind unten gegen die Schädelbasis zu durch eine schmale knöcherne Commisur in Verbindung." *This description is exactly correct for the adult* (Fig. III h), *but in the young there exists only an unmodified aperture in the frontal for the exit of this nerve.* Cope (14) attaches great importance to the free margin of the frontal bone in the adult. The frontal in the young has no such margin, the surface being slightly depressed in the middle and regularly convex laterally.

The frontals overlap the parietals to a considerable extent posteriorly. A cross-section through the posterior part of the parietal of the adult presents the curve of a quarter circumference, the depression being external for the temporo-cervical tendon. In the young this bone slopes from the median line outwards at an angle of thirty degrees, and is but slightly depressed in the middle of the distal half.

The orbito-sphenoid confirms Hay's account, being higher in front than behind. Its ossification is almost complete. The exoccipitals remain almost entirely cartilaginous, being invested with a thin parostosis. The ossification of the condyles is beginning. The pterygoid is represented by a bar of cartilage, free anteriorly but attached to the quadrate posteriorly. The otic capsule is quite surrounded by cartilage. The otolithic deposit is extensive. The proötic is cartilaginous to a considerable extent. The stapes shows no evidence of ossification, as is likewise the case with the columella. The quadrate

is perfectly formed in cartilage. The squamosal is the only otic element entirely ossified. A thick plate of basioccipital cartilage lies beneath the hinder portion of the brain. The parasphenoid forming the floor of the brain-case is somewhat convex in its anterior part, but markedly concave in the posterior region. The lateral walls of the brain-case in the pituitary region posterior to the orbitosphenoid are of cartilage, as in the adult. The other features of the cranium are so similar to the descriptions of Hay and Kingsley that I deem it useless to give them.

The Visceral Skeleton.

The mandible is quite the same as in the adult. The rami are united anteriorly by strong connective tissue. Meckel's cartilage lies in the groove of the ramus as far forward as the point just below the eye, and extends backwards so far as to be in contact with the quadrate. The dentary and angular are well ossified. The teeth are fully developed. The hyoid apparatus is unlike either the adult or Hay's embryo. It is for the most part cartilaginous. The middle third of the basibranchial appears quite well ossified. Thin parostoses invest the ceratohyal and ceratobranchial. The following elements compose the apparatus : one basihyal, two hypohyals, two ceratohyals, one basibranchial, two ceratobranchials, and eight epibranchials. Hay found no basihyal in his embryos. A distinct nodule of cartilage is present at the juncture of the hypohyal and ceratohyal on the external side. Its significance is unknown to me. The ceratohyals extend outwards rather than backwards, as in the adult. The larynx is an exceedingly simple structure, consisting of a fibrous connective tissue tube, strengthened by two lateral bars of cartilage. The external orifice is a longitudinal slit. The trachea has no rings or partial rings of cartilage.

Soft Parts of the Head.

The disposition of the muscles is approximately the same as in the adult, with the exception that the place of the temporocervical tendon appears to be filled by muscular tissue. Ven-

trally the first inscriptio tendinea is seen just below the basihyal. The remnants of some nasal glands are present along the olfactory sac. Hay found these glands better developed in his specimens. The most important and interesting structure is found below and external to the eye in my smallest specimen, seventy-eight millimetres in length. *There appears in this region a canal* (Fig. 15) *one-tenth of a millimetre long, which is walled by columnar epithelial cells extremely regular in outline. External to the epithelial wall there is seen a thick layer inferiorly of degenerated tissue, which is bounded by a thin layer of fibrous connective tissue. In three other specimens, eighty-eight, ninety, and ninety-two millimetres respectively, no trace of this degenerate canal could be discovered, and in the smallest specimen I was able to detect it on the right-hand side only.* Here it was very clearly seen, as shown in Fig. 15. The significance of this atrophied element will be discussed later. Hay (3), Kingsley (5), and Osborn (8) have to a limited extent described the nervous system of Amphiuma. Owing to the fact that my tissue was not prepared for the demonstration of the nervous elements, I can add only one or two points of interest. The brain (Fig. 14) is much shorter than in the adult, caused mainly by the wedging in of the middle brain between the hemispheres of the great brain. The brain viewed dorsally, excluding metencephalon, presents the outline of a longitudinal section of a hen's egg, the anterior end corresponding to the smaller end of the egg. The olfactory lobes are not marked off as in the adult. The brain of *Siphonops annulatus*, as figured by Wiedersheim, resembles to a considerable extent the brain of young *Amphiuma*. The latter, however, is not so elongated as the former. The pineal gland and pituitary body so prominent in the adult are scarcely distinguishable. Hay and Kingsley have described the origin and distribution of the cranial nerves sufficiently for our purpose. My sections clearly corroborate the statement by Hay that the facial nerve passes beneath the columella. Kingsley failed to find the roots of the fifth nerve. *My sections show but one root.* The dorsal ganglion in connection with the twelfth nerve is quite large, being at least one-third the size of the gasserian ganglion.

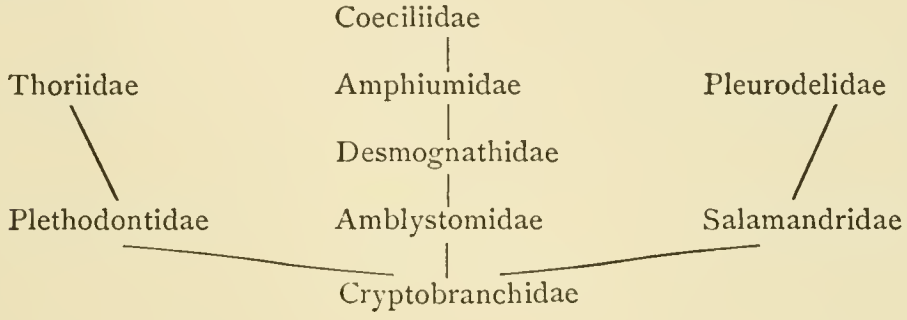
Axial Skeleton and Appendages.

The vertebrae are only partially ossified. The transverse processes are wholly cartilaginous, and a portion of the internal part of the body of the vertebrae is unossified. The cartilaginous posterior projection of the roof of the spinal cord is invested with a thin parostosis. The cartilaginous intercentra are present. The ribs are present as cartilage. The neural and diapophysial spines are imperfectly developed. The hypapophyses are well marked. The shoulder girdle is wholly cartilaginous, and presents elements of scapula, coracoid and precoracoid. The scapula is exceedingly thin, being only two cells in thickness. The other elements are correspondingly slender. No sternum is present. The humerus is covered with a thin layer of bone, except in the regions of the extremities. The radius and ulna are also ossifying externally. The carpus is present as pure cartilage, and the phalanges remain as hyaline tissue also. The pelvic girdle is entirely cartilaginous. All the elements are present as in the adult, but there is no evidence of the posterior bony disc. The femur is invested with a very delicate ectostosis in the shaft region. The future prominent trochanter process is indicated by a slight flexion of the cartilage at that point. The two abductor, the adductor and two rotator muscles have the same locations as in the adult. The tibia, fibula, tarsus, metatarsus, and phalanges show no signs of ossification. This affords weighty evidence that the formation of the anterior limbs is in advance of the posterior.

I have not been able to discover any conical arrangement in the fibres of the dorsal muscles. The abdominal muscles have the same relative dispositions as in the adult. The transversalis is unaffected by the inscriptiones tendineae, and arises from the internal plate of fascia, originating in common with the external plate on the neural spine. The digestive, respiratory and excretory systems correspond with those of the adult. Having described the anatomy of these different individuals, it remains to determine the genealogical status of *Amphiuma* among vertebrates.

Phylogenetic Conclusions.

Amphiuma has always been considered a degenerate form. Cope (4) says that *Amphiuma* is the annectant type which Wiedersheim sought for in tracing the ancestry of the *Coeciliidae* to the Stegocephali of the Carboniferous period, and then adds that he derives the *Coeciliidae* from the Urodela direct through the *Amphiumidae*, and adds the following table of affinity:



It is evident to all phylogenists that this table presents an absurdity, since representatives of each of the five families in the direct line of descent are existing at the present time. That these families are closely related cannot be denied. Cope bases his strongest point of relationship between *Coeciliidae* and *Amphiumidae* on the common possession of an ethmoid, when in fact the latter family does not possess an ethmoid. Sections of my young specimens clearly demonstrate this. *What Cope has called the ethmoid are merely the descending processes of the frontals.* Kingsley (5) believes that the many peculiar resemblances of *Gymnophiona* and *Amphiuma* are those of homoplassy. The recent information gained by the examination of the young specimens in my possession, enables me to prove that these resemblances are due, in part at least, to relationship. The *Ichthyophis glutinosus* of Ceylon, as described by the Sarasins (9), is undoubtedly closely allied to *Amphiuma*. It is now known that the eggs of the former are fertilized within the body of the parent. In my description of the reproductive organs of *Amphiuma* I have demonstrated that in this genus also internal fertilization takes place. Both deposit eggs of about the same size, which are united by a

twisted cord. Both incubate the eggs by lying in a coil about them. In their larval life the young of *Ichthyophis* possess gills and dwell in the water. Hay's embryos of *Amphiuma*, evidently near the period of hatching, had well developed gills. The young specimens I secured last February were found under rocks near the water, indicating that their transformation from aquatic to land habits had lately been accomplished. All these superficial features common to the two genera indicate affinity; but by far the stronger evidence of their affinity is based on the structure of the soft parts as well as the skeletal elements. The peculiar disposition of the fascial investment in *Amphiuma* is also seen in *Ichthyophis*. The dorsal lamina arising from the neural spines splits into two plates before reaching the lateral line, and one enters the body cavity to give rise to the transversus abdominis, which is unaffected by the inscriptiones tendineae probably on account of its late formation in the embryo. The omo-humero-maxillaris is absent in all urodels except the Coeciliidae and Amphiumidae. The lungs of *Amphiuma* are very unequal in length, a condition characteristic of the Coecilians, according to MacAlister. There is also a striking similarity in the trachea of the two families. Wiedersheim speaks thus of the Gymnophiona: "Die Luftröhre ist entsprechend den weit nach hinten gerückten Lungen für ein Amphibiem von sehr bedeutender Länge und componirt sich aus zahlreichen hyalinknorpeligen Ringen, welche dorsalwärts nicht geschlossen sind, sondern hier durch Bindgewebe ersetzt werden." As I have already shown, the trachea of *Amphiuma* is comprehended in the above description of a Coecilian. The brain of the young *Amphiuma* is much more unlike the brain of the adult than the latter is unlike the brain of *Siphonops annulatus*, as figured by Wiedersheim. The distribution of the cranial nerves in the two species is almost identical.

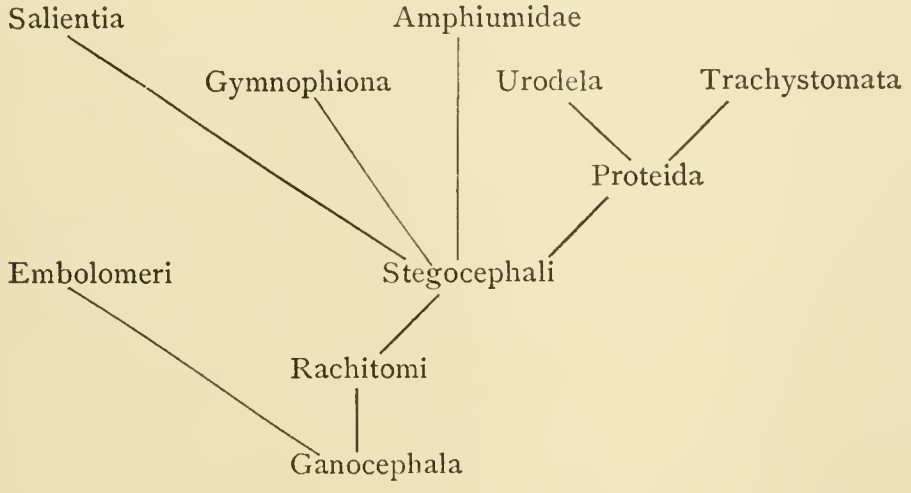
In *Siphonops annulatus*, *Coecilia rostrata*, *Coecilia oxyura* and *Ichthyophis glutinosus* Weidersheim has figured and described an orbital gland and peculiar tentacular apparatus of the nasal region. This apparatus consists of a canal beginning posteriorly to the eye, whence it extends forward to its external orifice

near the narial aperture, a tentacle or feeler, and a muscle by means of which the tentacle is retracted or protruded to guide the animal in its dark underground expeditions. *As I have already shown, there exists in my youngest specimen of Amphiuma the atrophied remnants of this tentacular apparatus. The columnar epithelial lining of the canal is very distinct in about one dozen transverse sections taken through the orbits. In some of the sections I have discerned what I believe to be the degenerated retractor muscle. This apparatus in Amphiuma has precisely the same relative location as in the Coecilians.* For some unexplainable reason, neither Hay nor Kingsley found this organ in the young embryo. Hay speaks of nasal glands, which, from his description, I conclude to be identical with the glands I noticed in conjunction with the olfactory cavities. The tentacular canal of *Amphiuma* cannot be mistaken for the duct of a nasal gland, as it lies too far lateralward and posterior to the nasal region. It will not answer for the duct of the orbital gland, as it is too far inferior to the orbit, although it is possible that its relation to the surrounding parts may have become somewhat distorted. However that may be, the occurrence of this degenerated structure in the young *Amphiuma* and its complete disappearance in the adult gives unmistakable evidence of the relationship of the *Coeciliidae* and *Amphiumidae.* The disappearance of the ethmoid in the latter family can be accounted for by the fact that the descending processes of the frontals have displaced the cartilage for the ethmoid so that it lies beneath the forebrain in my young specimens. The nodule of cartilage between the anterior ends of the vomeropalatines of the adult has no genealogical significance, so far as I can discover. As before stated, the dense connective tissue is being transformed into cartilage in my specimen of 68 mm. *The cause of this formation is found in the fact that the teeth of the lower jaw in biting do not meet the corresponding ones in front, but pass inside of them, pressing against the roof membrane of the mouth, thereby exciting the growth of the cartilage in the same manner as the horns originated in the Cavicornia and Cervidae,* according to Eimer (17). That the teeth in the adult *Amphiuma* bite anterior to the summit of the car-

tilage, is explained by the anatomy of the young, in which the lower jaw is relatively shorter than in the adult. A similar abbreviation of the lower jaw is exhibited by the majority of the *Gymnophiona*, as shown by Wiedersheim (Taf. V, Figs. 58 and 59).

The vertebrae of *Amphiuma* are highly specialized, having definite processes for the attachments of its complexly constructed trunk musculature. As Cope has already suggested, the prominent anterior hypapophyses are peculiar to the *Coecilians* and *Amphiuma*. Thus far I have pointed out the features in these two families which give evidence of genealogical affinity. The proof of relationship furnished is to my mind conclusive ; but the gravest question — what that relationship is — remains to be answered. If Huxley's dictum, "It is more important that similarities should not be neglected than that differences should be overlooked" were maintainable, near affinity of these two families must be admitted. Before such affinity can be asserted, important contrasts in skull structure must be explained. Thus Kingsley says : "The presence of an ethmoid in the *Gymnophioma* (and its absence from *Amphiuma* and other Urodeles), the existence of a turbinal, the absence of a parasphenoid, and the presence of a basisphenoid are all points of importance." Another striking contrast is seen in the structure of the orbit which is only partially encircled by the bony elements in *Amphiuma*, there being no jugal or quadrato-jugal bone present. Gervais gives a concise description of the coecilian orbit : "Cependant l'orbite des Cécilies n'est percée ni dans le maxillaire seul ni dans le corps de l'os jugal ; c'est ce que l'on voit très-bien sur la tête d'un jeune animal de ce genre; et avec quelque attention, surtout en se servant d'une loupe, on retrouve même chez l'adulte des traces de la suture des deux os entre lesquels l'œil est ici placé, et qui concourent, comme chez beaucoup d'autres animaux, à former un cercle orbitaire." These differences in skull structure make it patent that *Amphiuma* cannot be the connecting link between the leg-bearing Urodeles and the Coecilians, as Cope has asserted. The elongated cranium, the double series of teeth, the tentacular apparatus, the degenerated optic sense, the manner of

fructification and incubation of the eggs, the habits of the young, the degenerate limbs, the unusual disposition of the transversalis-abdominis, the inequality of the length of the lungs, the anterior hypapophyses and the amphicoelous vertebrae, all of which these two families possess in common, point to a common parent form of the *Coeciliidae* and *Amphiumidae*. The numerous differences in the skull structure of the two families make it manifest that the common ancestor is a form far back in Geologic time ; a fact which tends to verify Wiedersheim's statement that the origin of the *Coeciliidae* is to be sought in the Stegocephalans of the Carboniferous. The well-developed columella auris of *Amphiuma* is very probably a character retained from the *Ganocephala* and *Rachitomi*. In the light of present paleontological and embryological knowledge any detailed phylogeny of Amphibia must be very uncertain. However, the facts at hand seem to me of such significance as to warrant the following table : —



Although *Amphiuma* is considered a degenerate form, yet certain parts of its structure are highly specialized. The general shape of the cranium presents a marked contrast in comparison with other Amphibian skulls. The great length of the face and the pointed snout are of no phylogenetic significance, as they have been developed by the habits of the animal. The vertebrae with their numerous processes cannot be accounted for on any other ground than that of adaptation to the mode of life which rendered it necessary that a complex trunk

musculature should exist, and the required processes for the many attachments. The conical arrangement of fibres in the dorsal muscle reveals a condition quite the opposite of degeneration. The fact that similar muscular cones are found in the alligator (13) and *Sphaenodon* does not imply that these three forms are in any way related. The existence of an unusually long and strong temporo-cervical tendon in *Amphiuma* and *Desmognathus* does not furnish sufficient evidence that they are closely allied, as Cope has tabulated them. These are merely cases of parallelism, as is plainly shown when we take into consideration the marked contrast of the more important structural elements of the two families. Scott (19) has demonstrated this condition of parallelism in numerous mammalian families. "The prismatic, cement-covered molar has been independently developed in many forms: *e.g.*, several of the ruminants, certain pigs, the horses, one of the rhinoceroses (Elasmotherium), the elephants, many rodents, *etc.* The selenodont molar pattern has been several times independently evolved; (1) in the true ruminants; (2) in the camels; (3) in the oreodonts. The spout-shaped odontoid process of the axis has arisen in the true ruminants, the horses, the camels, and, to a certain degree, in the later oreodonts, such as Merychyus." Gegenbaur (20) [p. 669, Fig. 232] has described and figured incorrectly the muscular arrangement in the tail of the fish. The wall of the cone is incomplete adjacent to the spinal column, in all the fish which I have examined. This tendency toward conical arrangement of fibres in the tail of the fish has been evolved by the same mechanical principles that obtain in *Amphiuma*. Thus it may be noticed that in many respects *Amphiuma* is not a degenerate form, but on the contrary possesses highly developed structures; and were it not for the fact that the brain exhibits such primitive characters [Osborn (8), p. 178], I would consider this type the result of progressive rather than retrogressive evolution.

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EXPLANATIONS OF PLATES XXXIII AND XXIV.

FIG. 1. Dorsal aspect of Amphiuma's skull, X 2. P, premaxillary; *n*, nasal; *m*, maxillary; *f*, frontal; *pi*, parietal; *pf*, prefontal; *q*, quadrate.

FIG. 2. *p*, premaxillary; *m*, maxillary; *pd*, parasphenoid; *pt*, pterygoid; *q*, quadrate; *st*, stapes; *a*, exit for ninth and tenth nerves.

FIG. 3. Lateral view of premaxillary with nasal, maxillary, prefrontal, and portion of frontal removed, X 2. *a*, brain cavity; *g*, frontal bone; *h*, canal for exit of olfactory nerve; *i*, nasal septum of premaxillary; *b*, a break in the septum.

FIG. 4. Dorsal muscles of head. *f*, pterygo-maxillaris; *h*, masseter; *i*, cervico-parietalis; *d*, *e*, temporo-cervical tendons; *a*, frontal bone; *g*, proötic.

FIG. 5. Muscles on dorsal aspect of posterior limb. *c*, femur; *a*, rectus femoris; *e*, ilio-peroneal.

FIG. 6. Muscles on ventral aspect of posterior limb. *c*, adductor magnus; *e*, gracilis; *a*, inscriptiones tendineae.

FIG. 7. Great adductor muscle of posterior limb. *d*, femur; *h*, tibia; *a*, cartilaginous plate; *e*, trochanter; *g*, pectineus; *b*, ossified disc; *c*, pubo-tibialis.

FIG. 8. Transverse section of adult at the fortieth vertebra. *a*, left side; *i*, transversalis-abdominis; *h*, obliquus internus; *g*, obliquus externus; *k*, fascia; *e*, fascia split from external envelope.

FIG. 9. Incision through right body wall and both walls reflected. *l*, lateral fascia line; *c*, muscular cones.

FIG. 10. The three rows of cones seen when the skin and fascia are removed from right side of Amphiuma. *b*, triangular rod of fat lying above the vertebrae; *c*, tendinous cord connecting apices of cones; *a*, fascia of cone reflected to form an inscriptio tendinea.

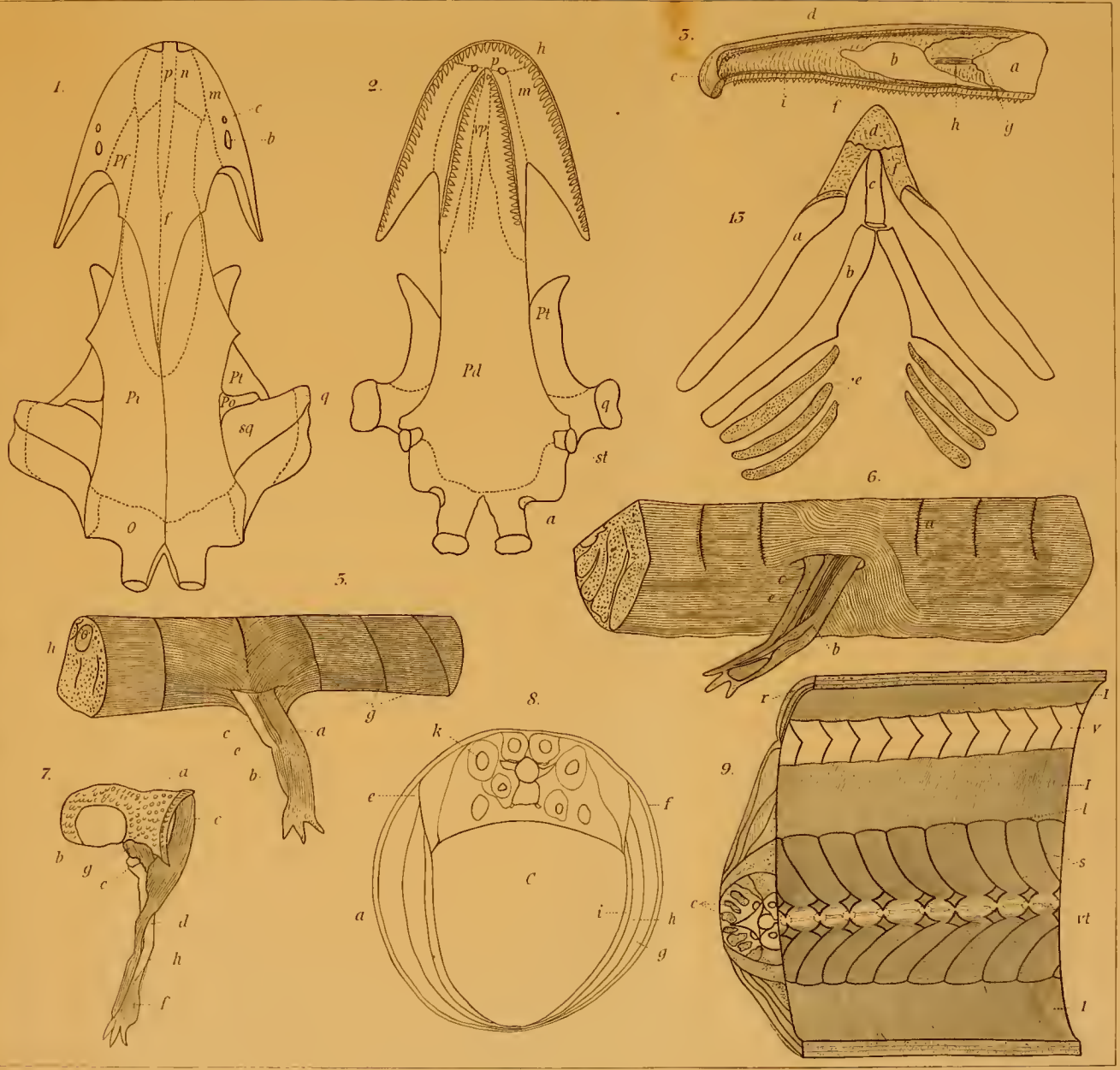
FIG. 11. Vent of the female longitudinally split open. *c*, capillary tubes; *b*, folds of membrane; *a*, entrance to the oviduct; *d*, lip of vent.

FIG. 12. Young Amphiuma, 78 mm. × 1½. *a*, gill opening.

FIG. 13. Hyoid apparatus of adult. *e*, epibranchialis; *b*, cerato-branchial; *c*, basibranchial; *d*, basihyal.

FIG. 14. Brain of young Amphiuma seventy-two millimetres long. *a*, *b*, prosencephalon; *c*, mesencephalon; *d*, metencephalon; *e*, spinal chord.

FIG. 15. Transverse section of the optic region of young Amphiuma sixty-eight millimetres long. *d*, eye; *r*, retina; *o*, orbitosphenoid; *p*, parasphenoid; *f*, atrophied tentacular canal; *e*, degenerated gland; *a*, brain; *m*, ramus; *n*, Meckel's cartilage; *h*, *i*, *k*, hyoid apparatus.



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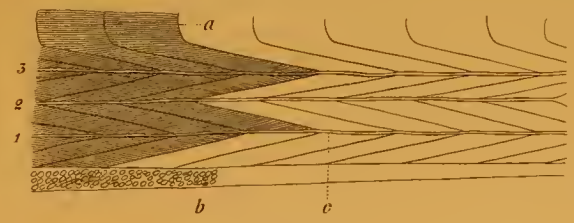

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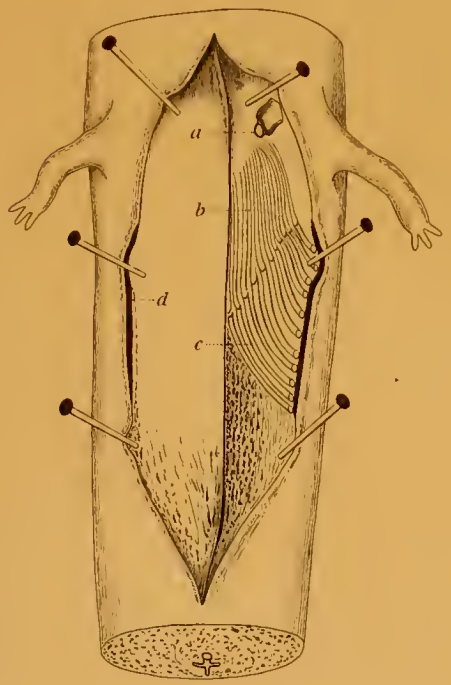

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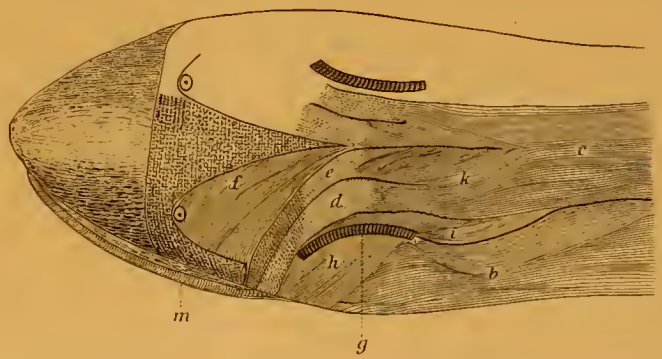

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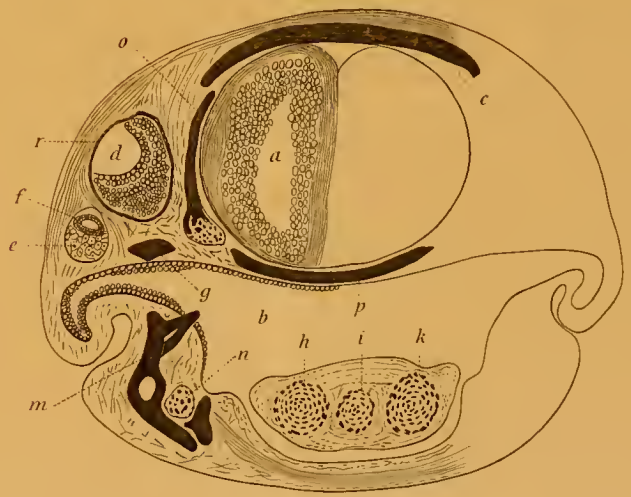

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ON THE ENTERON OF AMERICAN GANOIDS.

GRANT SHERMAN HOPKINS, B.S.

CONTENTS.

Historical Sketch.

The history of the successive discovery of facts relative to the morphology of the enteron in the vertebrates may be said to have undergone a process of evolution comparable to that through which the forms with which it deals have passed.

The first stage may be comprised between the years 1836 and 1869. "In 1836, Dr. Sprott Boyd (9),[1] in attempting to discover whether or not there existed an internal membrane of the stomach below that of the mucosa, failed to find what he sought, but saw the mucosa covered uniformly with small tubes, which he described and figured in several species of mammals, birds, and reptiles. The openings of these glands had been seen previous to the year 1836, and also isolated follicular or glandular masses had been noted, but Boyd was the first author who saw the mucosa of the stomach entirely covered with tubular glands from the cardia to the pylorus."

In 1838, Henle and Purkinje (51) each found that these glandular tubes were lined with cells; a year later, Wasmann

[1] See References.

(64) discovered in the pig the existence of two kinds of glands, — the gastric or peptic, and pyloric or mucous glands. During this period there was noticed in the gastric glands only one kind of element, large rounded and very granular cells, which Frerichs called pepsin cells. Of all the authors, including Kölliker (1856), Milne-Edwards (1859), Leydig (1866), and Klein (1870), who at this time gave attention to the structure of the glands in the fundus of the stomach, no one mentions any other kind of cell.

The second period in the growth of our knowledge concerning the fine anatomy of the enteron dates from the appearance of three articles by Rollet (55), Heidenhain (24), and Ebstein (17) in 1870, and continues to the year 1880.

Rollet distinguished in the glands of the stomach two kinds of cells, which he called "delomorphous" and "adelomorphous" cells. Heidenhain established the same distinction under the names of "Hauptzellen" and "Belegzellen" (principal and border or parietal cells), terms which were also employed by Ebstein, and which have come into general use. Ebstein devoted himself especially to the study of the pyloric glands, and so thorough was he in this that his descriptions and figures have been but little improved upon to the present day. "It was established from this time that in the mammals the base of the cardiac glands of the stomach was occupied by a covering formed of cylindrical cells more or less granular, the principal cells; that the upper portion was covered by very large cells charged with granules, the pepsin cells of Frerichs; and that in the middle of the tube the prismatic cells occupy the axis of the gland tube; the border cells were crowded to the outside, and determined by their exterior projection that bulging aspect of the isolated gastric glands, which one meets in all the previous accurately made drawings." (Pielliet.)

In the second edition of his *Traité d'Anatomie*, 1873, Sappey gave a morphological description of the glandular tubes in different kinds of vertebrates, but he failed to discern more than one form of cell in the gastric tubule; he says that they are formed of two tunics. The ectal, which is amorphous and homogeneous, presents no apparent difference in the two kinds of

glands. The ental tunic of the peptic glands is composed of large rounded cells which impart an irregular bulging form to the surrounding tunic; the pyloric or mucous glands are lined by small prismatic cells which do not reach to the center of their cavity.

One of the most important contributions that appeared during this period was an article by Edinger (18), in 1877, upon the mucous membrane of fishes. He says that the gastric glands appear phylogenetically first in the class fishes — the Selachians. Consequently the older vertebrates, like the invertebrates, have no specially differentiated portion of the alimentary tract for the purpose of digesting fixed bodies, albumen substances, *etc.* As to the presence of a stomach in the Dipnoans he is in considerable doubt, but seems to think it rather doubtful if they possess one. Hyrtl, he says, could find no trace of gland openings in the stomach of Lepidosiren paradoxa. In Protopterus, Parker (47) says: "The whole of the mucous membrane of the stomach and intestine is perfectly smooth, and there is no indication of any differentiated gastric or intestinal glands. Cilia are present on the epithelium throughout the stomach and intestine." Among the Teleosts, according to Edinger (18), there are several that have no stomach glands. They are absent in Cobitis fossilis, Gasterosteus pungitius, Tinca vulgaris, Abranus barbio; according to Rathke (53), they are wanting in Blennius ocellatus and Sanguinalentus, Gobius melanostomus, Cyprinus chrysophrasius, and Atherina Boyeri. Probably they fail also in Balistes.

In all these there is found a single somewhat granular cylindrical epithelium without beaker-cells. In such animals digestion must be performed, in part at least, by an intestinal secretion. The gastric glands have probably developed from the ordinary insinking of the alimentary epithelium. Their ontogenesis in the higher vertebrates as well as in those of lower rank indicates the above mode of formation, as shown by Kölliker, Barth, and Laskowsky. In Mammals, Birds, and the Batrachians it has been proved that the epithelium of the stomach glands, at an early stage of their development, is uniform throughout, and only at a later period are those cells

situated in the fundus of the crypts transformed into gastric cells.

The differentiation of cells into principal and border cells does not obtain in fishes; whether it occurs in Amphibia is doubtful. Heidenhain and Trinkler (61) found in the frog only one kind of cell; Edinger, however, found in the frog cells which gave as sharp color differentiation as one usually finds between the principal and border cells in Mammals and Birds. In Necturus maculatus the examination of several specimens by myself failed to show border cells. In an investigation on the enteron of Necturus by Dr. B. F. Kingsbury[1] no parietal cells were found.

The last stage of advance in our knowledge of this subject comprises the period from 1880 to the present time. During this epoch investigations have been directed principally to the determination of the evolution and mode of regeneration of the glandular epithelial cells and to their physiological significance.

No one, perhaps, has done more towards the solution of these problems than Dr. Nichola Trinkler. His paper (61), "Ueber den Bau der Magenschleimhaut," is devoted almost exclusively to the consideration of the epithelial cells and their transformations. As the result of various experiments and investigations, he concludes that the parietal cells increase in number during digestion, and that the young cells which arise from them by fission move gradually towards the lumen of the gland, and are there transformed into principal cells, and in this manner serve to replace the destroyed principal cells.

From experiments with artificial digestion he shows that the parietal cells of higher vertebrates and also the gland cells of lower forms (Frog, Pike) secrete pepsin, but that the presence of the parietal cells accelerates two or threefold the rapidity of digestion of fibrin and egg-albumen.

Langley (32), in his paper, "On the Histology and Physiology of the Pepsin-forming Glands," confined himself principally to the granules of the gland cells and to changes which

[1] The Histological Structure of the Enteron of Necturus maculatus. Proc. Amer. Micro. Soc., Vol. XVI, pp. 18–64, 1894.

occur in them during digestion and hunger. His observations were made upon certain Amphibia and Reptiles.

From the study of mitosis of the epithelial cells, Bizzozero (6) concluded that the cells did not live and die in the place where they originally arose, but that by degrees the deeper-lying cells of the epithelium reached the free surface in a manner precisely comparable to that which takes place in the epidermal cells of the skin. In certain glands which had attained their full development he found numerous nuclear figures, premonitors of active cell multiplication. These newly-formed cells, he says, gradually replace the epithelial cells of the free surface of the stomach, which, in certain animals, is in the highest degree desquamous. In a more recent paper (7), in which he examined the enteric glands and epithelium of the mouse, he arrived at the same conclusion as above, namely, that in the mouse, as in the rabbit, the gland epithelium is gradually transformed into the surface epithelium of the mucous membrane.

Pilliet (49), in a paper on the evolution of the glandular cells of the stomach, says that the gastric glands may assume very different appearances, and that by noting the form and structure of the glandular cells at various points of the gastric follicles he has tried to form a general idea, by studying the cells from their appearance to their death, where each of the different conditions were to be fixed in chronological order, and to indicate the particular age to which it corresponds in the life of the cell. He reaches the same conclusion as Bizzozero in regard to the gradual metamorphosis of the glandular into surface epithelial cells.

Other writers on this subject might be cited, but enough has already been said to indicate something of the nature of the work already done, and the forms which have been studied. Comparatively little has been done on the enteron of some of the lower or more generalized forms. Among the fishes or fish-like vertebrates very little attention has been given to that old and interesting group, the Ganoids. Indeed, in the literature that I have been able to examine, only the most meager references are made touching the morphology of the enteric

glands and epithelium of this group, and those by only a few observers. To certain of the Ganoids no reference whatsoever was found on this subject.

Material and its Preparation.

The forms upon which this paper is based are as follows:

Family Acipenseridae.

Acipenser rubicundus. (Common Lake Sturgeon.) *Scaphirhynchops platyrhynchus.* (Shovel-nosed Sturgeon.)

Family Polyodontidae.

Polyodon folium ? (Spoon-billed Sturgeon, Duck-billed Cat.)

Family Lepidosteidae.

Lepidosteus osseus. (Gar-Pike, Bony Gar, Bill-Fish.)

Family Amiidae.

Amia calva. (Bowfin, Dog-Fish, Mud-Fish, Lawyer, Johnny Grindle.)

The material was obtained and placed in the hardening fluid immediately on the spot where the various forms were taken. Specimens of the common sturgeon, Polyodon, and Scaphirhynchops were obtained in the month of February (or the very first of March) from Knoxville, Tennessee. Other specimens of Polyodon and those of Lepidosteus were taken in the latter part of August from the Mississippi River at Ft. Madison, Iowa.

The material was preserved by hardening in picric alcohol (95% alcohol, 1 part; water, 1 part; picric acid, $\frac{1}{5}$%) from one to two days; then it was placed in 67% alcohol for a day, after which it was kept in 82% alcohol. When needed for use, small pieces from various regions of the enteron were dehydrated in 95% alcohol for one day, then soaked in chloroform twelve to twenty-four hours, after which they were infiltrated and embedded in paraffin. Mercuric chlorid was also used as a hardening agent with very satisfactory results. The fresh tissue was placed in the solution of mercury ($HgCl_2$, 5 grams; NaCl, ½ gram; H_2O, 100 cc.) for one-half to two hours — a

longer time seems to do no harm. From this the tissue was transferred to 70% alcohol to which a little tincture of iodine had been added. The tissue is washed in this till the alcohol ceases to lose color; then it is transferred to 75% alcohol for a day, after which it is dehydrated and embedded either in paraffin or collodion.

The cilia of ciliated epithelium are perfectly preserved, so far as can be judged, by each of the above-named hardening agents.

Various stains were tried, but the most satisfactory combination was haematoxylin and eosin. For separating the muscular coats 20% nitric acid was used. The tissue was left in the acid until it had sufficiently dissolved the connective substance to permit the ready separation of the fibers. This is effected, usually, in from one-half to two days, depending largely upon the temperature, the acid acting more rapidly in a warm atmosphere. When the tissue has been sufficiently macerated, further action of the acid is prevented by placing the tissue in a saturated aqueous solution of alum, plus 2% chloral hydrate, in which it may be kept for an indefinite period. This is also a good method for isolating the gastric glands. For isolating the epithelial cells Müller's fluid, picric alcohol, 30–35%, and alcohol, 30–35%, were used; of these Müller's fluid was the most satisfactory.

It is a source of regret that the material could be examined in a fresh condition only in the cases of Amia and Lepidosteus.

The outlines of the figures were made by aid of an Abbé camera lucida; the details were put in freehand.

Common Lake Sturgeon.

General Form of Enteron. — The conformation of this portion of the abdominal viscera is somewhat complex. From the mouth the enteron extends nearly one-half the length of the abdominal cavity, where it doubles upon itself and ascends several centimeters in front of the opening of the pneumatic duct. The ascending portion is somewhat to the left of the descending part; the intervening space is filled by a lobe of the liver. At the point above indicated the ascend-

ing part curves upon itself to the right, and extends backward about two-thirds the length of the abdominal cavity, where it changes its direction and ascends to a point a little cephalad of the middle of the cavity; here it again forms a loop, and thence extends directly to the vent. From this description and the diagram (Fig. 1) it will be seen that there are three descending and two ascending parts to the enteron. The oesophagus and stomach are almost wholly included by the two first portions, and it is with these that this paper has chiefly to do. At the pyloric end of the stomach, in an adult sturgeon, the walls attain a thickness of from two to four centimeters. Immediately following this thickened part are the pyloric caeca. These appear like a single organ, but from the occurrence of three distinct openings leading into the mass, as Ryder (56) says, it must be regarded as a "system of pyloric caeca three in number"; the tubes soon subdivide into a number of small branches analogous to a racemose gland. The remainder of the enteron will be passed over by simply remarking that the walls appear somewhat thin, and that at the terminal end of the intestine the spiral valve makes seven complete turns, the last one extending nearly to the vent. The peritoneal coat has a uniformly dark, almost black, appearance, due to the deposition of pigment (Fig. 5). Within the peritoneum is the thin longitudinal muscular coat; the great bulk of the muscular tissue, however, is comprised within the circular layer. Along the oesophagus this layer is very thick, and the striated fibers are grouped into large fascicles. In the gastric region the striated muscular fibers are superseded by the unstriated fibers; the circular layer is thinner, and the fascicles are smaller and more compact. No oblique muscular coat was observed.

The various regions of the enteron are not sharply differentiated from each other. In a general way the subdivisions can be recognized, but the boundary line between oesophagus and stomach, for instance, cannot be determined by the gross appearance of this region. A part, at least, of that portion which most observers have called oesophagus ought, it is believed, to be regarded as really a part of the stomach.

Oesophagus.— In his work on the "Fishes of France," Moreau (41) says of Acipenser: "The oesophagus is covered or armed with papillae more or less conical and directed backwards. The stomach is scarcely larger than the oesophagus; it would be difficult to establish the line of separation if the gastric mucosa did not present a different appearance, it being entirely smooth. It is at the commencement of the stomach, a little back of certain small pad-like structures formed by the termination of the oesophagus, that the canal of the air-bladder opens." Ryder recognizes in the alimentary canal three very clearly defined regions. Of these the first, or oesophageal, "extends as far back as the opening into the air-bladder. The gullet proper, . . . upon being laid open, is found to be covered for some distance with backwardly-directed soft fleshy processes, into which its mucous membrane is elevated. At some distance in its course farther back, its lining membranes again become smooth, but slightly folded longitudinally." With but one or two exceptions all the authors mentioned in this paper, who have expressed themselves on the point, state that the pneumatic duct opens into the oesophagus. The epithelium of the latter, according to Leydig (33) and several others, is like that of the buccal cavity, a stratified pavement epithelium. So far as noted, all authors say that there are no glands in the oesophagus. From the above quotations it will be seen that the stratified pavement epithelium extends backward to the opening of the pneumatic duct, and that this region is non-glandular. The correlation of the parts, as found by the writer, is somewhat at variance with the above statements. In my specimen, an adult sturgeon about two metres (six feet) in length, the stratified epithelium, together with the fleshy papillae, disappear at a point about 5 cm. in front of the pneumatic duct opening. Succeeding the stratified is a columnar epithelium which extends uninterruptedly to the pylorus. Considered from a mechanical standpoint, almost every one would say that the stratified should overlap the columnar epithelium, but the exact reverse is the case. At the transition point the stratified epithelium becomes obtusely wedge-shaped; the deeper layers are overlaid by cylindrical-shaped cells whose

length varies in conformity to the inclined surface covered by them (Fig. 7).

In the writer's opinion this mode of transition may be regarded as a type to which, it is believed, most if not all of the various groups of vertebrates will be found to conform.

Edinger, whose investigations included Cyclostomes, Selachians, one Ganoid (Lepidosteus), and many Teleosts, makes this statement: "Where pavement epithelium is present, it becomes thinner and thinner towards the stomach, the interspersed beaker-cells increase in number and soon form a continuous stratum which extends over one or two layers of the flat cells, but at the border of the stomach lies directly upon the connective tissue of the mucosa."

In certain mammals (dog, cat) and in one reptile (soft-shelled turtle) the transition from stratified to columnar epithelium has been found to correspond to the above type, Gage (21). Extending from this point nearly to the pylorus, the epithelium is ciliated (Fig. 12). For a short distance immediately beyond the point of transition the mucous membrane appears very similar to that at the pyloric end of the stomach; the epithelium is infolded, forming deep crypts, or follicles, which closely resemble the pyloric glands; in the former, however, the epithelial cells are ciliated, while in the latter they are not. The first few crypts are lined by columnar ciliated cells only, but the true glandular cells make their appearance before we reach the opening of the pneumatic duct. In the latter case the tubes are lined by two kinds of cells, the glandular cells occupying a short segment at the base, and the ciliated cells the remaining portion (Fig. 6). In many cases two or more glands open into a common outlet. From the above facts the writer concludes that the caudal portion of that segment of enteron which most writers have called oesophagus is in reality a portion of the stomach, a conclusion substantiated by Gegenbaur (22) and Milne-Edwards (39).

Stomach. — Although the stomach of certain of the sturgeons has been investigated to some extent, no reference has been found in the literature on the forms relative to an interesting morphological feature found in the present species.

Ciliated epithelial cells have been found in the oesophagus of sturgeons, but no one mentions their presence in the stomach, and several deny their ever existing there.

Concerning the glands in the stomach of the sturgeon Leydig says: "They are short cylindrical sacs . . . lined with great regularity by a clear and delicate cylindrical epithelium, which is continuous at the edge of the gland orifice with that of the surface epithelium; the cells of the two cylindrical epitheliums are distinguished in this, that in the surface epithelium they are larger and distended towards their free extremity by a molecular mass." If the above statement applies to all the glands of the stomach, Leydig's specimen differed greatly from the one studied by me. It has already been noted that in my specimen the greater part of the gastric epithelium is ciliated. Among the ciliated cells are numerous beaker-cells, many of which are open at the free end. The glands of the stomach are of the two ordinary kinds, cardiac and pyloric; the latter occupy but a small area compared to the former. The cardiac glands are differentiated into two very clearly recognizable portions, a superficial or mouth part and a deeper or body portion. The relative length of the two parts through the middle portion of the stomach is as 1 to 3 nearly (Fig. 9).

On both sides of this area the glandular cells are gradually replaced by those of a more nearly cylindrical form, till finally the tubules are lined by the latter form of cells only. In all the tubules, so far as observed, except those in the pyloric region, the mouth portion is lined by ciliated columnar cells (Figs. 6, 8, 9). No difference was detected between these and the cells of the surface epithelium except that the latter are longer and their attached ends do not end so abruptly. Beaker-cells are found in the mouths of the glands as well as on the free surface. The nuclei of the epithelial cells stain deeply with haematoxylin; the cell-body stains sparingly with eosin unless the stain is left on for some time; this applies equally well to the gland cells proper. The part of the cell next to the so-called basement membrane stains more deeply than the part next to the lumen of the gland. All the cells in the glandular portion of the tubule have approximately the same

form, — irregularly cylindrical or cubical-shaped; those next to the ciliated cells of the mouth may perhaps be somewhat more nearly cylindrical.

The attached ends of most of the glandular cells are continued out into a sort of sheet-like prolongation which appears to anchor the cells in place. Along the middle of the gland the cells overlap each other in a manner very similar to the scales of a pine cone, the apex of the cone corresponding to the fundus of the gland. From numerous instances in which the cells were traced directly into the mucosa it is believed by the writer that in the Ganoids, at least, a basement membrane does not exist. In Edinger's description of the stomach glands in fishes he says that they possess no membrana propria or basement membrane, but that the epithelium borders directly upon the connective tissue of the mucosa. The cell threads lie over each other like tile, so that they surround the upper part of each gland with a kind of membrane which is composed of innumerable fine threads, and sharply fixes the boundaries of the gland. This appearance is obtained only in the cardiac glands. In the pyloric region the glands are lined throughout by cylindrical cells; in these glands no cilia were found. "The differentiation of parietal cells, so far as is known, does not occur in the class fishes, but is a phylogenetic occurrence which appears much later in the vertebrate series." (Edinger.)

Scaphirhynchops.

Owing to lack of material, almost nothing can be said regarding the form and appearance of the enteron in this genus. The stomach is recurved upon itself in a manner similar to that of the common sturgeon. At the pyloric end the muscular walls are thickened, but not to such a marked degree as in the preceding. To all appearances the pyloric caeca are like the common sturgeon's. The peritoneal coat is unpigmented. In the oesophageal portion of the enteron are numerous large fleshy papillae; these disappear some distance in front of the pneumatic duct opening. In a specimen whose enteron, when straightened, measured about 15 cm. from the air-duct to the caeca, the papillae extended only to within about 3 cm. of the

pneumatic opening. The epithelium of this region is stratified; the surface layer is composed chiefly of large beaker-cells almost bladder-like in form; the nuclei are quite small and crowded down close to the attached ends of the cells. Near the place where the papillae disappear the stratified is succeeded by a ciliated columnar epithelium. The arrangement of the cells at the point of transition of the two epitheliums is as in the preceding form, *i.e.*, the ciliated cylindrical cells overlie the deeper layers of the stratified oesophageal epithelium as in Fig. 7.

The segment of enteron between this point and the opening of the pneumatic duct is, from the presence of glandular cells, regarded as the cephalic part of the gastrium. The stomach glands of the individual examined were not so long as those in the sturgeon; the comparative length of the mouth and glandular portion was, however, about the same proportion, 1 to 3 nearly (Fig. 13).

The mouths of the glands, except the pyloric, are lined by ciliated cells resembling those of the surface epithelium (Fig. 16). Among the ciliated epithelial cells are many greatly distended beaker-cells whose contents are coarsely granular and very sharply differentiated from the neighboring cells (Fig. 16). The granulation extends into the cell to the level of the nucleus. In some instances the granular mass could not be seen projecting beyond the level of the free ends of the cells, but in others this was noticed. Probably in the former the theca had not yet ruptured and the contents exuded as in the latter. The nuclei of the great majority of these cells were situated at a higher level than the others, as if the swelling caused by the granular accumulation within had started the cells somewhat from their normal position. Although these two kinds of cells are so different in appearance, it is not easy to determine in precisely what this difference consists.

Trinkler thinks that the only difference between beaker-cells and the ordinary cylindrical cells is, that in the former the metamorphosis of the protoplasm is more complete; he considers the beaker-cells as simply a later condition of the cylindrical cells.

The bodies of the gastric glands are made up of irregularly cubical-shaped cells which overlap each other as in the sturgeon. The cells are granular in appearance; the nuclei are large, and contain a distinct nucleolus. That portion of the cell next to the lumen of the gland stains much less deeply than the basal half of the cell. Usually more than one gland opens in a single mouth; the usual number was two, but frequently three or more were noted. The glands lie in close proximity to each other, there being but little intervening connective tissue.

In surface sections, *i.e.*, sections at right angles to the long axis of the gland, it was noticed that frequently several glands were united, as it were, into a bundle; the connective tissue surrounding these forming a thicker layer than around the individual glands. As seen in cross-section, the number of epithelial cells of a gland varies from eight to twelve in most instances (Fig. 14).

POLYODON.

The appearance of the enteron in this genus differs somewhat from either of the preceding. Its general outline is shown in Fig. 3. The papillae at the cephalic end of the oesophagus are small and numerous; they extend to within about one or one and one-half centimeters of the pneumatic duct. The walls of the stomach are considerably thicker than in either of the preceding specimens. At the pyloric valve the muscular walls are thickened as in Scaphirhynchops. The pyloric caeca are relatively much larger and more deeply subdivided (Fig. 3, *c*). The caecal cavity communicates freely with that of the intestine; it subdivides into four main branches, corresponding to the four main lobes into which the gland is divided. The intestine is short, morphologically speaking, but the spiral valve, with its six complete turns, really forms a long intestine, in a physiological sense. The last turn of the valve is about two centimeters from the vent. The peritoneal coat is almost entirely unpigmented, there being only a few small pigment patches.

The mode of epithelium transition and the presence of follicles, or crypts, in front of the pneumatic duct opening corresponds to those forms already noted, except that the follicular area is considerably shorter than in either of the preceding. The epithelium of the cardiac portion of the stomach is ciliated (Fig. 21). Owing to the shortness of the cilia and the presence of a thin layer of extraneous matter on the surface of the epithelium, considerable difficulty was experienced in detecting the cilia in this specimen. Among the ciliated epithelium cells were a great many beaker cells; the peripheral end of these takes scarcely any stain, and appears to be open, or without a membrane over the free end. The nuclei of the cells are very large, some oval and others circular in outline, and many of them contained several darkly stained granules. The cells lining the mouths of the glands are much shorter than those on the free surface, but nevertheless they possess fully as large nuclei as the latter; in other respects no differences between the cells in the two situations were observed. In this genus the mucous membrane presents certain features not found in any of the others. The gastric glands are so convoluted that it is almost impossible to get sections in which the whole length of the gland appears; usually the glands are cut at various angles to their long axis, so that perhaps two or three successive sections must be examined in order to study the gland throughout its whole extent. The relative lengths of the mouth and body of the gland are nearly equal (Fig. 18). The lumen of the glands was specially large in the specimen examined, and appeared to be of about the same size at all points except the mouth, which, of course, was somewhat larger. In several instances it was noted that the diameter of the lumen measured at least one-quarter the whole width of the gland (Fig. 20). Several glands open into a single mouth. The gastric cells which line the body of the glands have, at their attached ends, a very slight thread or sheet-like continuation for attaching them to the mucosa. The tile-like overlapping of the cells is very slight; often the dividing line between two cells extends directly across at right angles to the long axis of the gland. The cells are finely granular and stain lightly, especially the

part next to the lumen of the gland; the nuclei are large, either oval or circular in section, and situated close to the attached ends of the cells. In the fundus of the glands the nuclei, especially those of oval outline, appear to be undergoing division (Fig. 20). In many of the cells there may be seen extending across the short diameter of the nucleus a very deeply stained band of chromatin; in these cells no nucleoli can be seen. In the cells whose nucleus is circular in outline but one nucleolus is present, as a rule, but sometimes two or more were seen. In several instances nuclei were found somewhat constricted in the middle, and still others where two distinct nuclei were in direct apposition, each being somewhat smaller than the original parent nucleus from which, to all appearances, the two nuclei had been formed.

This appearance of nuclear division in the fundi of the glands is in perfect accord with the statements of several authors who have shown that in the adult of certain mammals the centers of growth of the enteric epithelium are situated in the fundus of the glands. If this is true of fishes, it would be interesting to know the exact concomitant changes which the cell must undergo in the process of its transformation from a non-ciliated glandular cell into a ciliated epithelial cell. The glands are quite widely separated from each other by the intervening mucosa, more so than in any of the other forms. The diameter of the glands is also less than in the two preceding forms, but this may be simply an individual variation. The pyloric glands are lined by narrow cylindrical cells, among which occasionally were seen greatly distended, coarsely granular beaker-cells. No cilia were found in this region, either in the glands or on the surface. The thickness of the stomach walls was previously alluded to; it is due in great part to the excessive thickness of the submucosa, which forms a layer as thick if not thicker than that formed by the muscular coats.

LEPIDOSTEUS.

In a specimen measuring 55 centimeters from the tip of the snout to the tip of the tail the enteron extends in a direct line

backward for a distance of 18 centimeters, where the first refolding occurs: the other flexures are indicated in Fig. 2.

There is no line of demarcation between oesophagus and stomach unless, as stated by Balfour and Parker (3), "a glandular posterior region be regarded as the stomach, a non-glandular anterior region forming the oesophagus." The macroscopic appearance of this region gives no indication of the position of the boundary line between the two parts. The intestine is of about the same size in all parts. The pyloric caeca are so small and numerous that the caecal mass formed by them presents an almost brush-like appearance. The caecal cavity extends into the finest subdivisions of the gland. The peritoneal coat is unpigmented. The short spiral valve makes only two or two and one-half turns, and ends about two centimeters from the vent. In connection with this part of the intestine is a structure referred to by Balfour and Parker as follows: "The posterior part of the intestine, from the beginning of the spiral valve to the anus, is connected with the ventral wall of the abdomen by a mesentery. . . . This mesentery, which together with the dorsal mesentery divides the caudal section of the body into two lateral compartments, is, we believe, a persisting portion of the ventral mesentery which, as pointed out by one of us,[1] is primitively present for the whole length of the body-cavity. The persistence of such a large section of it as that found in the adult Lepidosteus is, so far as we know, quite exceptional. . . . The small vessel in it appears to be the remnant of the subintestinal vein." My specimen agrees perfectly with the above statement; the ventral edge of the mesentery in this specimen measures at least 5 cm. in length. The blood-vessel, supported by the mesentery, divides into two nearly equal branches, one of which extends forward and the other backward, to be distributed to the abdominal parietes. No reference to a ventral mesentery in any of the other ganoids has been noticed, and the writer has not had opportunity of examining other forms than Lepidosteus and Amia. No ventral mesentery was found in Amia.

The papillary structures found in the oesophagus of the

[1] *Comparative Embryology*, Vol. II.

three preceding forms do not exist in Lepidosteus, owing, possibly to the great development of the dental armament, which practically precludes the escape of prey, when once fairly within the mouth, without any intervention of subsidiary structures.

In Lepidosteus[1] the non-glandular cephalic portion of the enteron extends from the pneumatic duct caudad a distance of 8 to 10 cm. before the gland structures of the stomach appear. This part, which will be called oesophagus, is covered by a ciliated columnar epithelium interspersed with numerous beaker-cells (Fig. 17). In front of the pneumatic opening the epithelium is stratified, being composed principally of large mucous cells, but among these are a considerable number of cylindrical and fusiform cells. Owing to the rounded form of the beaker or mucous cells, the nuclei, situated close to the attached end of the cells, presents a disk-like or saucer-shaped appearance. Of the epithelium of the stomach of fishes Edinger says (Ueber die Schleimhaut des Fischdarmes nebst Bemerkungen zur Phylogenesis der Drüsen des Darmrohres, p. 666), "Cilia, where such cover the mucous membrane of the oesophagus, disappear in the stomach. . . . The epithelium of the stomach is a cylindrical epithelium which never bears cilia." Possibly these statements are true for all the specimens he examined, but the probabilities are that he overlooked the cilia in the stomach of Lepidosteus. The grounds for this belief are, first, he himself found ciliated epithelial cells in the oesophagus of Lepidosteus ; and second, as already stated, the oesophagus in the present specimen is ciliated, but ciliated cells were found in the stomach as well. Possibly the reason for Edinger not finding them was due to defective preservation of the cilia in this region. Cilia were not found over the whole extent of the cardiac region, but were confined to its cephalic part. The ciliated cells did not form a continuous layer but were more or less scattered among the nonciliated epithelial cells of this region.

The epithelial cells are long and slender; the thread-like

[1] Size of specimen not known, but the hardened specimen of oesophagus and stomach measured somewhat over twenty centimeters.

ends can be traced down among the connective-tissue corpuscles of the mucosa; not the slightest indication of a basement membrane was noticed. From many of the beaker-cells of the oesophagus can be seen conical or rounded projections of mucus, but, unlike Scaphirhynchops, these mucous masses are stained very little. The transition from oesophagus to stomach is gradual. Towards the gastric end of the oesophagus the epithelium gradually infolds, forming short, broad follicles. These are soon replaced by the true gastric glands of the stomach. The latter are lined by large granular, irregularly cubical-shaped cells, except the part near the exit of the gland upon the surface, where the cells are of a broad cylindrical shape, but there is no sharp line of demarcation between the two. The glands of Lepidosteus, unlike those of the other forms, have, for the most part, no portion lined by cells of the same size and appearance as those of the surface epithelium. The part corresponding in position to the mouth is lined by cells two or three times as large as those of the surface. The relations of the cells at this point are such as to give the appearance of the gland having been forced up through the surface epithelium like a wedge (Figs. 23 and 26).

As a rule the glands open singly upon the surface, but sometimes two or more have a common outlet. The cells along the upper half of the glands show clearly the thread-like continuations extending from their basal end; in the deeper part of the gland they are somewhat shorter. The nuclei of the gland cells are circular, those of the surface epithelium oval in outline. No indications of cell division were noted.

As we approach the pylorus the glands grow shorter; at the same time the upper part becomes lined with cells like those of the surface epithelium, till finally the glands are lined with columnar cells only; these glands are considerably shorter than the cardiac glands. In the cardiac region the glands are close together, and of about the same diameter at all parts (Fig. 26). The lumen is distinct and enlarged somewhat just before it opens upon the surface. Cilia were not found in any part of the lumen of the gland in any portion of the stomach.

Amia.

In this, the most teleostoid in appearance of Ganoids, one might, perhaps, expect to find morphological features unlike those of the other members of the group. Macroscopically, the enteron of Amia does differ to a certain extent from the others, but microscopically the resemblances are very close. But little, if any, taxonomic value can be ascribed to this organ so far as could be ascertained from the individuals examined. The general form of the enteron in Amia is shown in Fig. 4. The chief differences between this and the preceding are that in Amia the gastric portion of the enteron is, comparatively, very much enlarged and of somewhat different shape, and there are no pyloric caeca. The spiral valve makes four or four and one-half turns, the last ending a little more than a centimeter from the vent.

In the adult specimens examined there was no distinct ventral mesentery connecting this part of the intestine with the ventral abdominal wall as in Lepidosteus. Papillae were not found back of the pharyngeal dental pads. The fibers of the circular muscular coat are striated as far as to the place where the glands of the stomach first appear, just caudad of the pneumatic duct opening; elsewhere unstriated fibers were found. The pneumatic duct opens near the junction of the oesophagus and stomach. Between oesophagus and stomach is a short region occupied by rather short, broad follicles lined by columnar ciliated cells; the true gland cells are first seen at a point a little beyond the opening of the pneumatic duct.

According to Schulze (58) the epithelial cells of the stomach in all vertebrates are open, *i.e.*, the free ends of the cells are not covered by a cell wall.

The mucus which these open cells secrete, Edinger thinks, is for the purpose of protecting the cells themselves from the digestive action of the secreted fluids. Brinton (11) also seems to hold the same opinion. He says: "The protection of the stomach from its own secretion is effected mainly by the salivary and other secretions which enter it from the oesophagus and the duodenum. . . . For units of mucous membrane,

fishes seem to have the most powerful gastric digestion." To the writer these opinions appear unsatisfactory from the fact that in the American Ganoids, at least, the ciliated character of the gastric epithelium would tend to prevent the formation of a distinct mucous coat over the surface of the stomach. But apart from this, it is believed that the vital properties of the cells are sufficiently potent to withstand any deleterious effects which the gastric secretions might possibly have upon them. Edinger thinks that the functions of the mucus are to thin the chyme and to form a protective covering over the hard, indigestible bodies, as sand, shells, *etc.*, which find their way into the stomach. He says that such foreign bodies, surrounded by a tough mass of mucus, are frequently found in the intestine. This explanation seems more reasonable than the preceding.

Ebstein found open as well as closed cells, and is of the opinion that during digestion the membrane of the closed cells is ruptured. In all the specimens examined by me both open and closed cells were found. The epithelial cells of Amia's stomach are very slender (Fig. 28), and the attached ends are continued into long thread-like processes which intertwine with the subadjacent mucosa. Ciliated cells are found uninterruptedly from the cephalic end of the oesophagus to within two or three centimeters of the pylorus. Scattered among these were many open beaker-cells, — the two kinds of cells being in about equal numbers. From the open end of many of the beaker-cells a mucous mass of varying size was seen projecting a variable distance beyond the free end of the cells (Fig. 27). At the cardiac end of the stomach the gastric glands appear as short tubes at the base of the follicles above mentioned. They, however, rapidly increase in length, and over the middle portion of the stomach make up the greater part of the tubule (Fig. 25). As the pyloric region is approached, the glandular part decreases in length, and about two centimeters from the pylorus disappear. From this point on, the glands are lined with cells like those forming the surface epithelium, only shorter. In the cardiac region the mouths of the glands are short, and are lined by ciliated cells (Fig. 24). The cells of

the body of the gland are, for the most part, cubical in longi-section of the gland, but for a short distance below the mouth the cells are more nearly cylindrical in outline. In Fig. 24 it will be noticed that the cells lining the mouth of the gland are placed obliquely to its long axis. In all the forms examined this arrangement holds true, only to a more marked degree than there represented. Frequently cells were seen so bent that the angle formed equaled at least a right angle. In all cases the convexity of the cells projected towards the exit of the gland; the attached end of the cells reached a much lower level than the opposite end.

In the pyloric region the glands are quite widely separated from each other; the lining cells of these are situated at nearly right angles to the long axis of the gland. Towards the pyloric valve the glands become shorter and gradually disappear, or pass into the crypts of the intestine. Cilia were not found in these glands or on the surface epithelium.

Summary.

The salient points of this paper may be summarized briefly as follows:

1. The gastric glands were first discovered by Sprott Boyd in 1836. Two years later Henle and Purkinje each discovered that the glands were lined with cells.

2. In 1870 Rollet distinguished in the glands of the stomach two kinds of cells, which he termed "delomorphous" and "adelomorphous" cells. Heidenhain, in the same year, noted the same distinction under the names of "principal cells" and "parietal cells."

3. Phylogenetically, the gastric glands first appear in the Selachians.

4. The ontogenetic development of the glands indicates that they have developed from simple insinkings of the alimentary epithelium.

5. The differentiation of parietal cells does not occur in the class fishes, and it is doubtful if it does in the Amphibia.

6. The centers of multiplication of the enteric epithelial cells appear to be at the fundus of the glands.

7. The boundary line between oesophagus and stomach can be determined only by microscopical examination.

8. In the sturgeon, Scaphirhynchops, Polyodon, and Amia deep follicles are found cephalad of the pneumatic duct opening. In Lepidosteus the pneumatic duct opens very far in front of the place where the follicles first make their appearance. In sturgeon and Scaphirhynchops true gastric cells (as inferred from their form and appearance) are present cephalad of the pneumatic duct; that is, to all appearances, in these two last named forms the pneumatic duct opens into the stomach.

9. The typical form of transition from the stratified epithelium of the oesophagus to the columnar epithelium of the stomach is illustrated in Fig. 7. The columnar and stratified epitheliums are wedge-shaped at the place of transition, the columnar overlying the stratified epithelium.

10. A basement membrane could not be demonstrated in any of the individuals examined.

11. The ontogenetic development of the glands shows that primitively they were lined throughout their whole length by cells like those of the surface epithelium. The true glandular cells are a later specialization; hence those glands with a comparatively long mouth and short glandular portion are regarded as more primitive than those with a short mouth and long glandular portion.

12. From the above statement we conclude that the gastric glands of Lepidosteus are somewhat more highly specialized than those of Amia, and that both these are more highly developed than in any other members of the order.

13. Pyloric caeca are present in all except Amia; a spiral valve is present in all, being most rudimentary in Lepidosteus.

14. Ciliated cells were found in the oesophagus and stomach of all the members of the group examined.

15. Ciliated cells were not found in the pyloric region of the stomach, either on the free surface or in any portion of the glands.

16. The significance of cilia in the enteron is doubtful. Trinkler thinks they have not much meaning; he regards them

as residual structures of the embryonic period. Doubtless they are of little importance. Possibly they facilitate the dissemination of the gastric juice and other fluids secreted by the stomach.

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EXPLANATION OF PLATE XXV*a*.

All the figures of this plate except 2, 3, and 4 are from the common sturgeon.

FIGS. 1–4. Diagrams of the enteron:

FIG. 1. Common sturgeon. *A* pyloric caeca.

FIG. 2. Lepidosteus. *B* pyloric caeca.

FIG. 3. Polyodon. *C* pyloric caeca.

FIG. 4. Amia.

FIG. 5. Pigment cells in the peritoneum of the enteron.

FIG. 6. Gland from near the cephalic end of the stomach showing the long upper portion of the tubule lined by columnar ciliated cells and the short glandular portion at the base.

FIG. 7. This shows the mode of transition from the stratified epithelium of the oesophagus (or pharynx) to the ciliated columnar epithelium of the succeeding part.

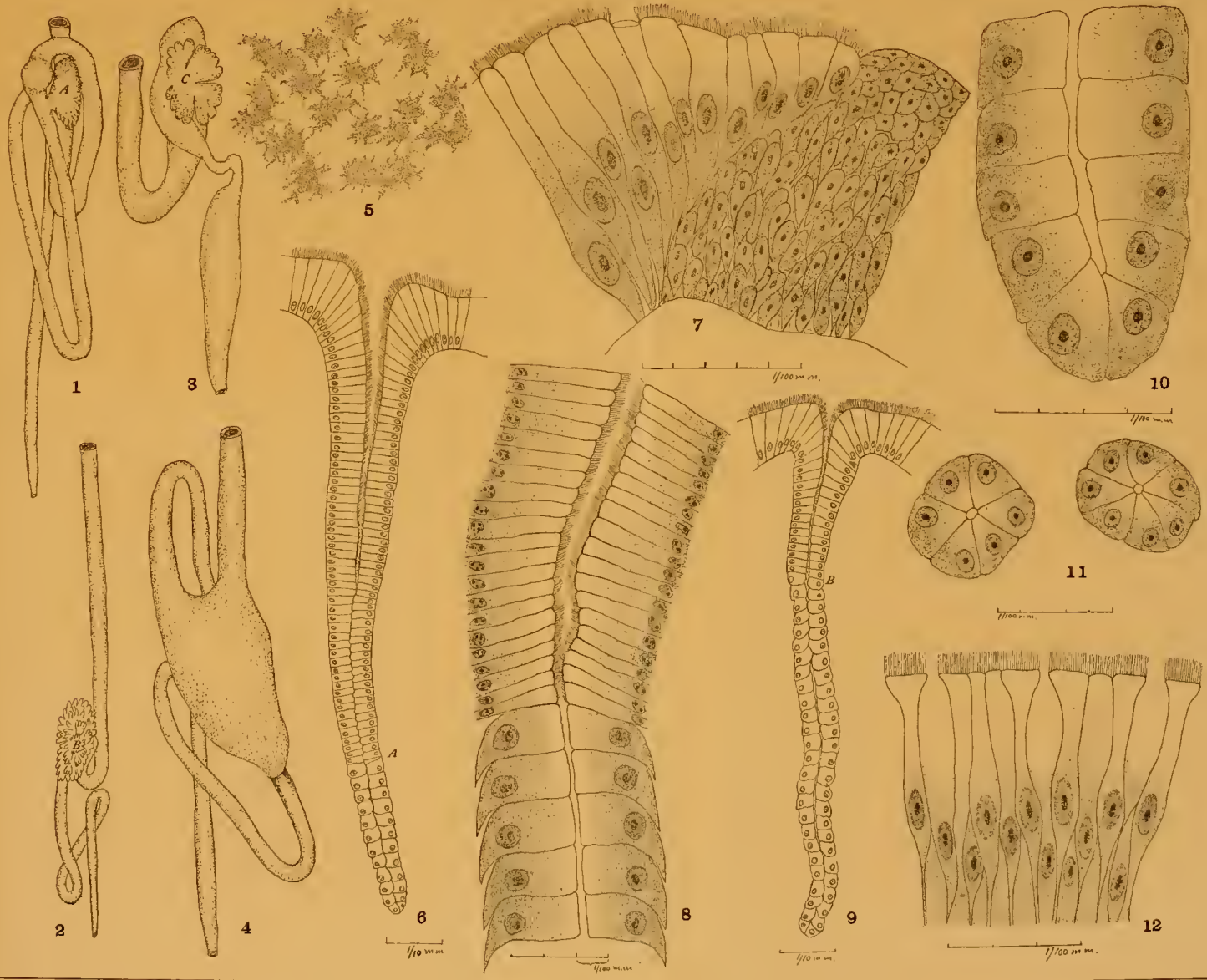
FIG. 8. Enlarged view of Figs. 6 and 9 at points *A* and *B*.

FIG. 9. Gastric gland from near the middle of the stomach.

FIG. 10. Longisection of gastric gland.

FIG. 11. Transection of the same.

FIG. 12. Ciliated epithelium of the stomach.


A
C
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3
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11
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EXPLANATION OF PLATE XXV*b*.

FIG. 13. Two gastric glands opening into a single mouth. (Scaphirhynchops.)

FIGS. 14 and 15. Transection and longisection of gastric gland of Scaphirhynchops.

FIG. 16. Enlarged view of Fig. 13 showing the ciliated character of the cells in the mouth portion of the gland. *A*, greatly distended beaker-cell.

FIG. 17. Epithelium from the oesophagus of Lepidosteus showing ciliated cells and beaker-cells.

FIG. 18. Gastric glands of Polyodon.

FIGS. 19 and 20. Transection and longisection of gland (Polyodon). *A*, nucleus undergoing division.

FIG. 21. Epithelium from the stomach of Polyodon showing ciliated cells and beaker-cells.

FIG. 22. Transection and longisection of gastric gland (Amia).

FIG. 23. Mouth of gastric gland, of Lepidosteus, showing the relation of the cells of the gland to the ciliated epithelial cells on the surface of the stomach.

FIG. 24. Mouth of gastric gland of Amia, showing the ciliated cells lining the mouth and also a few beaker-cells.

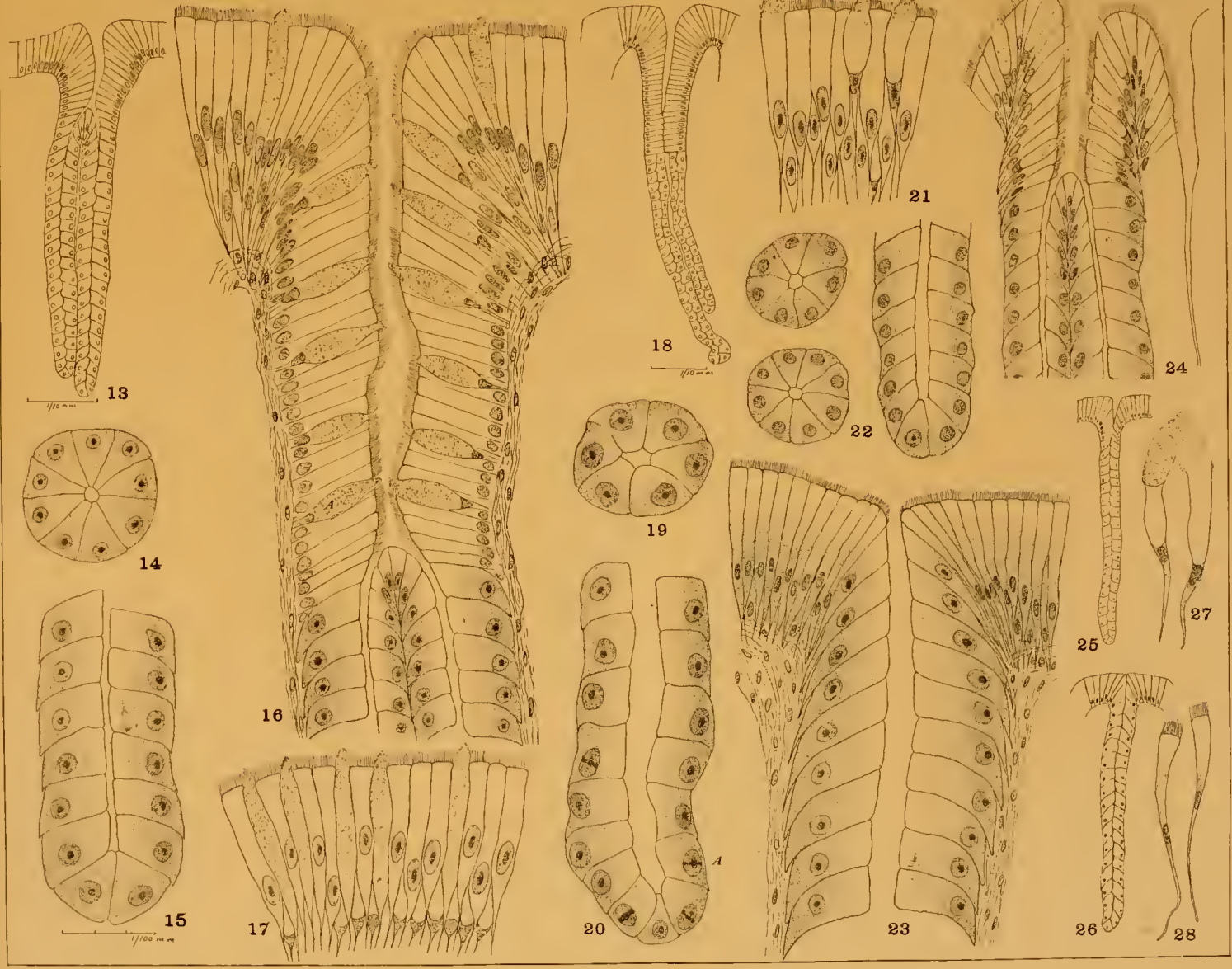
FIG. 25. Gastric gland (Amia).

FIG. 26. Gastric gland (Lepidosteus).

FIG. 27. Two beaker-cells from the stomach of Amia.

FIG. 28. Ciliated epithelial cells from stomach of Amia.

(Figures 18, 25, and 26 are on the same scale; all the others of this plate, except Fig. 13, are on the same scale as Fig. 15.)



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ARCHOPLASM, CENTROSOME, AND CHROMATIN IN THE SEA-URCHIN EGG.

EDMUND B. WILSON.

In a short paper published in January, 1895,[1] I showed that the fertilization of the egg in the sea-urchin *Toxopneustes variegatus* Ag. differs radically from Fol's account of the "Quadrille of Centers" in *Strongylocentrotus lividus*, and at the same time A. P. Mathews announced results nearly identical with mine in the case of the sea-urchin *Arbacia punctulata*, and the star-fish *Asterias Forbesii*. Since the appearance of that paper Boveri has published the results of an independent investigation of the fertilization of *Echinus microtuberculatus*,[2] which, as far as the essential phenomena of fertilization are concerned, agree "fast Punkt für Punkt" with those of Mathews and myself, — a confirmation which seems to render the evidence against the "quadrille" nearly conclusive as far as the echinoderms are concerned.

On two points only is there an apparent conflict between Boveri's results and my own. One of these, as Boveri himself points out, is merely a difference of interpretation, or rather of terminology, since the large reticulated spherical body forming the central mass of the asters of the karyokinetic figure ("*astrosphère*" of Fol, "*centrosphere*" of Strasburger) is called a *centrosome* by Boveri, whereas I termed it the "attraction-sphere" or "archoplasm-sphere," and asserted the absence of a "differentiated centrosome" (*l. c.*, p. 326).

The second point appears at first sight to be a difference of fact, since Boveri describes the sperm-aster as containing from the first a minute deeply staining centrosome of the usual type, whose division into two precedes the fission of the sperm-aster.

[1] Journal of Morphology, X, 1, p. 319.

[2] Ueber das Verhalten der Centrosomen bei der Befruchtung des Seeigel-Eies, nebst allgemeinen Bemerkungen über Centrosomen und Verwandtes. *Verh. der Phys.-Med. Ges. Würzburg*, N.F., XXIX, 1, 1895.

I shall endeavor to show, however, that even this difference, if it really exist, is probably of minor importance. The main purpose of this paper is, however, to record certain observations upon the structure and origin of the archoplasmic structures in *Toxopneustes*, and their relation to the centrosome and the chromatin, that diverge to some extent from Boveri's, and lead to a conception different from his.[1]

I. The Archoplasm.

Boveri's paper raises questions of the greatest interest regarding the relation of the archoplasm to other constituents of the cell, and they are of special importance, since he still maintains on the whole his original views, based on the study of *Ascaris*, which are opposed to those of Heidenhain, Reinke, and a number of other recent workers. Since it is to Boveri that we owe both the conception of the archoplasm and its name, it may be useful to recall briefly the statement of his original views and their recent modification.

As first employed in the second of the classical "cell-studies" the term "archoplasm" was applied to "that substance of the cell which at the time of division forms the achromatic nuclear spindle and the two polar astral systems" (*l. c.*, p. 61). On the basis of Van Beneden's discovery and his own that the centrosome persists throughout the "resting stage" of the cell, and may therefore be considered as a permanent cell-organ, he developed the archoplasm-conception clearly and logically as follows. The archoplasm is a specific substance, distinct from the remaining cell elements (p. 62), which in the resting cell may be scattered through the cell-substance or may be aggre-

[1] The accompanying photographic illustration and most of the text-figures are selected from a much larger series now in course of publication as an "Atlas of Fertilization and Karyokinesis of the Ovum" by the Columbia University Press (Macmillan & Co., N. Y.), to whom my thanks are due for permission to reproduce them here. Most of the observations here recorded were made (like the photographs) on sections of eggs fixed in sublimate-acetic and stained on the slide with iron hæmatoxylin, followed in many cases with Congo red or acid fuchsin. The sections thus obtained are of brilliant clearness and are far superior to those prepared by other methods tested (sublimate, Hermann's fluid, chrom-acetic, picro-acetic, picro-osmic, picro-sublimate).

gated into a spherical granular mass (equivalent to the "attraction-sphere" of Van Beneden), as the result of an attraction exerted upon it by the centrosome (p. 70). The entire achromatic division figure (amphiaster) is derived from this mass, which divides into two, while from each of the resulting products rays grow out into the cell-substance, some of them becoming attached to the chromosomes and forming the spindle-fibres, while others give rise to the radiating astral fibres (p. 81). At the close of the karyokinesis the archoplasm-fibres are again withdrawn into the central mass, breaking up into granules meanwhile (p. 128), so that each daughter-cell receives one-half of the entire archoplasmic material of the mother-cell.

Boveri was careful to guard against the view that the archoplasmic rays extend themselves during the formation of the amphiaster by progressive differentiation at their free ends, out of cell-material other than the specific archoplasm-material preëxisting as such. He believed, on the contrary, that he had obtained conclusive proof that the rays were formed exclusively out of the substance of the archoplasm-sphere (p. 79), and even went so far as to accept the probability that the archoplasmic microsomes, out of which the rays are formed and into which they again are resolved at the close of karyokinesis, possess a distinct morphological individuality that is never lost throughout the entire cycle of cell-division (p. 80).

The same general conception is adhered to in the recent paper on the sea-urchin cited above (p. 443), but with one important modification. *Ascaris*, namely, is now admitted to be an exceptional case, and Boveri is inclined to the view (which, however, is very cautiously expressed) that in most cases the archoplasmic fibres ("Radien") may be "ganz neue Organisationen" — "die aus dem Substanzengemenge des Protoplasma gleichsam auskrystallisieren" (*l. c.*, p. 40). However this may be, the asters cannot be regarded as arising by the morphological rearrangement of a preëxisting reticulum or alveolar structure as maintained by Bütschli, Heidenhain, Reinke, Eismond and other authors; and in the case of *Ascaris*, at least, Boveri finds himself justified in the positive assertion that the granular archoplasm-sphere and the aster derived from it are

entirely independent of the "gewöhnliche Fadenwerk der Zellsubstanz."

My own observations compel me to differ widely from Boveri on this point, and lead to a view which on the whole agrees with that of Heidenhain and Reinke, though differing from both in some important particulars. In *Toxopneustes* there is the strongest evidence that the astral rays developed about the middle-piece of the spermatozoön arise by a direct transformation or morphological rearrangement of the preëxisting cyto-reticulum, and that they grow at their outer ends, as the sperm-aster moves through the egg-substance, by progressive differentiation out of this reticulum. The spindle-fibres are formed in like manner out of the intra-nuclear achromatic network (linin); and my observations show conclusively, I think, that at the close of karyokinesis the greater part of the spindle-fibres are resolved into that portion of the general cyto-reticulum that lies between the two daughter-nuclei and takes no part in the formation of the succeeding amphiaster.

My observations indicate, furthermore (though the evidence is not conclusive), that the intra-nuclear network (linin) from which the spindle-fibres arise is in large part derived directly from the chromatin. If the latter view be well founded, a direct morphological continuity can be traced between chromatin, linin, archoplasm, and the cyto-reticulum.

Observations.

A. *Origin, Structure, and Growth of the Sperm-aster.* — The entrance and rotation of the sperm-head have been sufficiently described in my former paper.[1] A few moments after entrance, in favorable sections, the middle-piece may be distinctly seen as a rounded body lying at the base of the sperm-nucleus, from which a broad granular entrance-cone extends outwards towards the periphery, as in Fig. I, *A*. At this period no radiations are present in the surrounding cytoplasm, which has the appearance of a reticulum along the fibres of which are scattered minute granules or "microsomes." These, like those of

the entrance-cone, stain intensely blue in iron-hæmatoxylin, while the clearer substance in which they lie takes the hæmatoxylin very slightly, but is colored red by Congo red.

The first astral rays become visible when the sperm-head has rotated about 90 degrees, though in rare cases they may appear before the rotation has begun. In its first stages the aster seems to be nothing more than a slight radial arrangement of

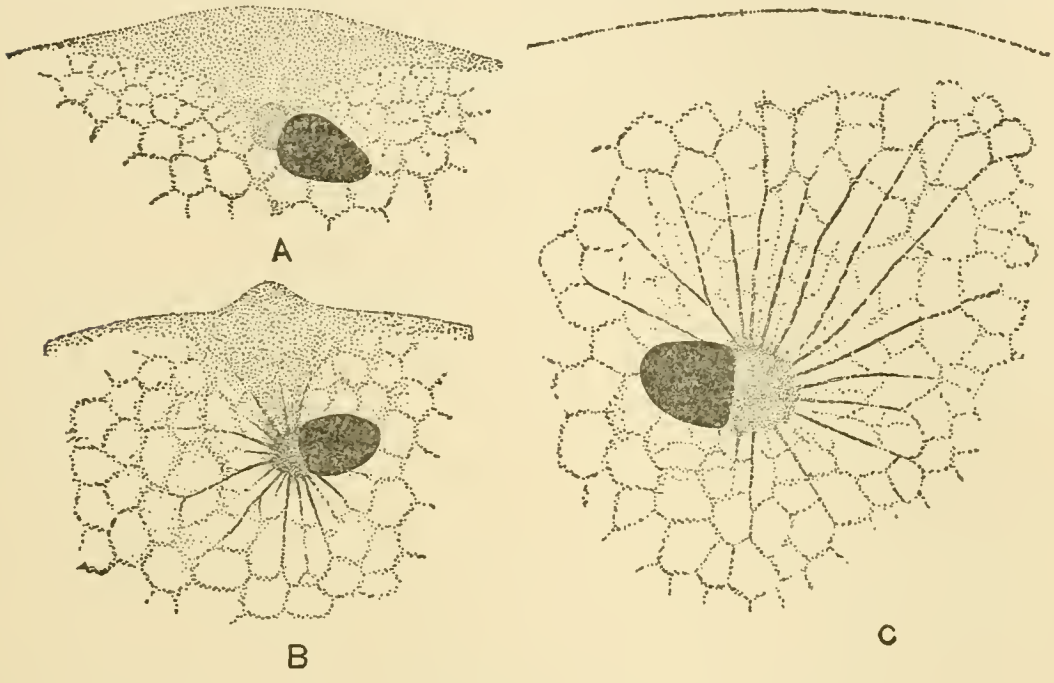


FIG. I. — Three successive stages in the formation of the sperm-aster. From sections.

A. Sperm-head soon after its entrance, showing the entrance-funnel, the cyto-reticulum composed of minute microsomes, the rotating sperm-nucleus and the spherical middle-piece at its base.

B. Later stage, showing the entrance cone and funnel, the advaucing rotation and the astral rays in process of formatiou out of the reticulum.

C. The sperm nucleus and aster shown in Prototype 2, drawn on a larger scale, showing the relation of the rays to the reticulum. (3 minutes.)

the mesh-work about the middle-piece (Text-fig. I, *B*). The latter may from this time forwards be called the central mass. As the aster increases (Phototype 2, Text-fig. I, *C*) the rays are clearly seen to be differentiated out of the reticulum, passing out into its meshes, where their outer ends become, as it were, lost in its substance. Some of the rays extend outward into the entrance-funnel, and may be traced in some cases actually into the entrance-cone.

At a first examination the rays have the appearance of continuous fibres; when closely examined, however, in the

most favorable specimens, they are found to be granular in structure as if composed of a string of beads.[1] At their base this appearance is less obvious, and sometimes cannot be distinguished ; but as they pass outward, the granular structure becomes more obvious, until at their tips, where they branch out into the general reticulum, they become quite indistinguishable from the latter (Fig. I, *B*, *C*).

As the pronucleus advances the rays rapidly extend themselves, and may be traced far out into the egg-substance, where they are lost in the general reticulum as in the earlier stages. The central mass meanwhile greatly increases in size (Phototype 3). Upon coming in contact with the egg-nucleus the central mass of the aster flattens somewhat against it and then rapidly extends itself so as to lie like a cap upon one side of it. The mass thus formed is of a horse-shoe shape in section, with a somewhat irregular contour (Phototype 4, Text-figs. II, III), from which radiate extremely long and conspicuous rays throughout nearly an entire hemisphere of the egg. The structure of the rays at this period is essentially the same as that in the earlier stages, but may be even more clearly made out. At their base they pass directly into, and are lost in, the central mass. At their outer ends they break up into the general reticulum. Careful examination shows further that they branch in their middle portions so that the number of rays continually increases towards the outer portion of the aster as described long since by Van Beneden. In their middle portions they have a wavy or sometimes even a zig-zag course, and the branching of the rays seems always to take place from one of the angles. Toward their bases the rays often appear as quite continuous fibres ; in their middle portions they are less regular and sometimes beaded in appearance ; toward their outer portions they often show in the clearest manner that they are composed of rows of blue-staining granules closely similar to those of the general reticulum, but as a rule somewhat smaller.

B. *Fusion of the Nuclei and Fission of the Aster.* — The

[1] *Cf.* Van Beneden and Neyt, Nouvelles recherches, etc., *Bull. de l'Acad. Roy. de Belgique*, III, 14, 1887, p. 266.

detailed history of the chromatin will be considered under another head, but a brief statement regarding the nuclei will be useful here. After coming into contact with the egg-nucleus

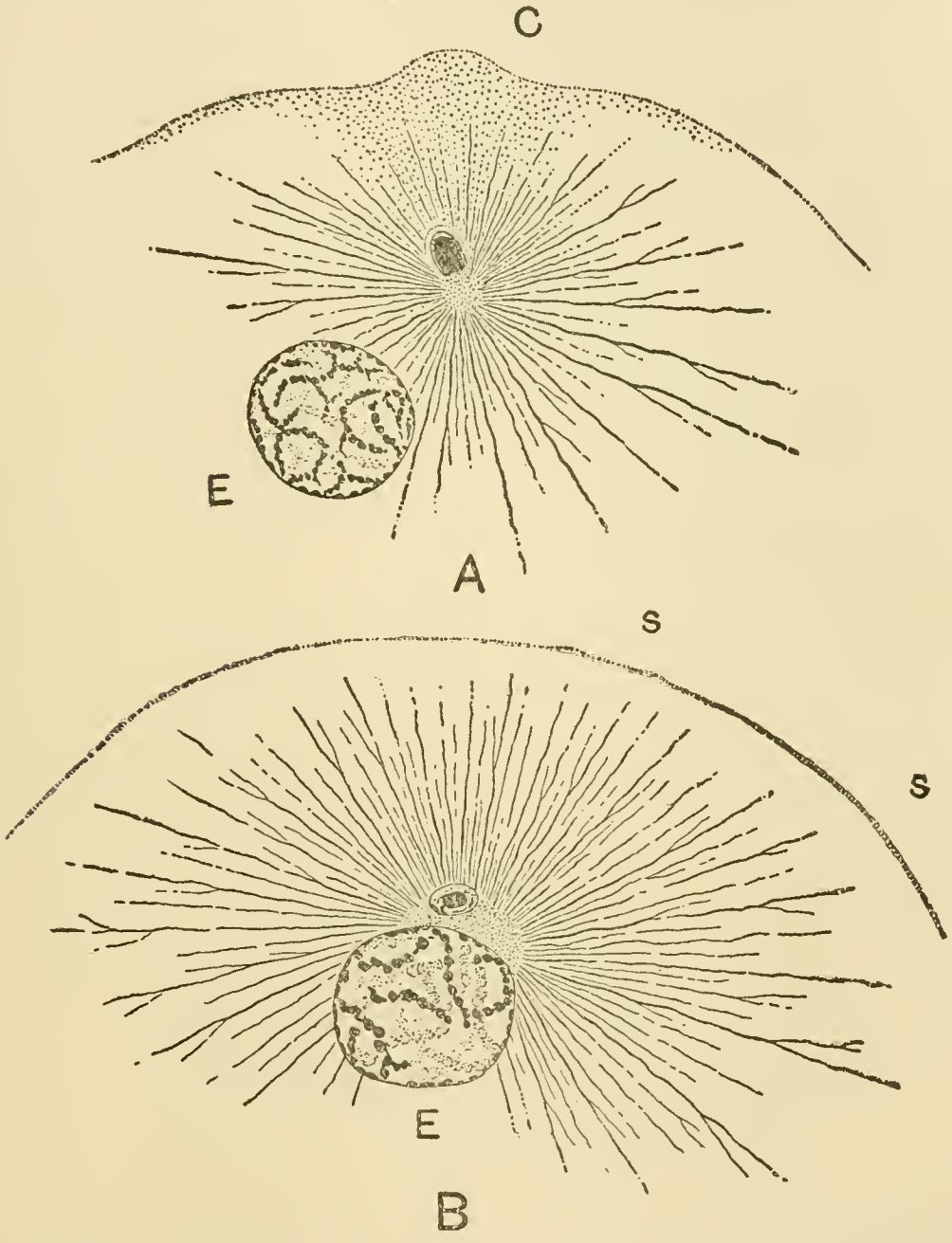


FIG. II. — Approach of the germ-nuclei showing the growth of the sperm-nucleus and aster and the branching of the rays. [*cf.* Phototype 3.] (6–8 minutes.)

the sperm-nucleus rapidly enlarges, its substance becomes reticular, and it assumes a vesicular form. It then flattens down closely against the egg-nucleus and assumes a lens-shape ; the boundary between it and the egg-nucleus then fades away,

and the substance of the two nuclei completely fuses, so that not the least visible distinction between the paternal and

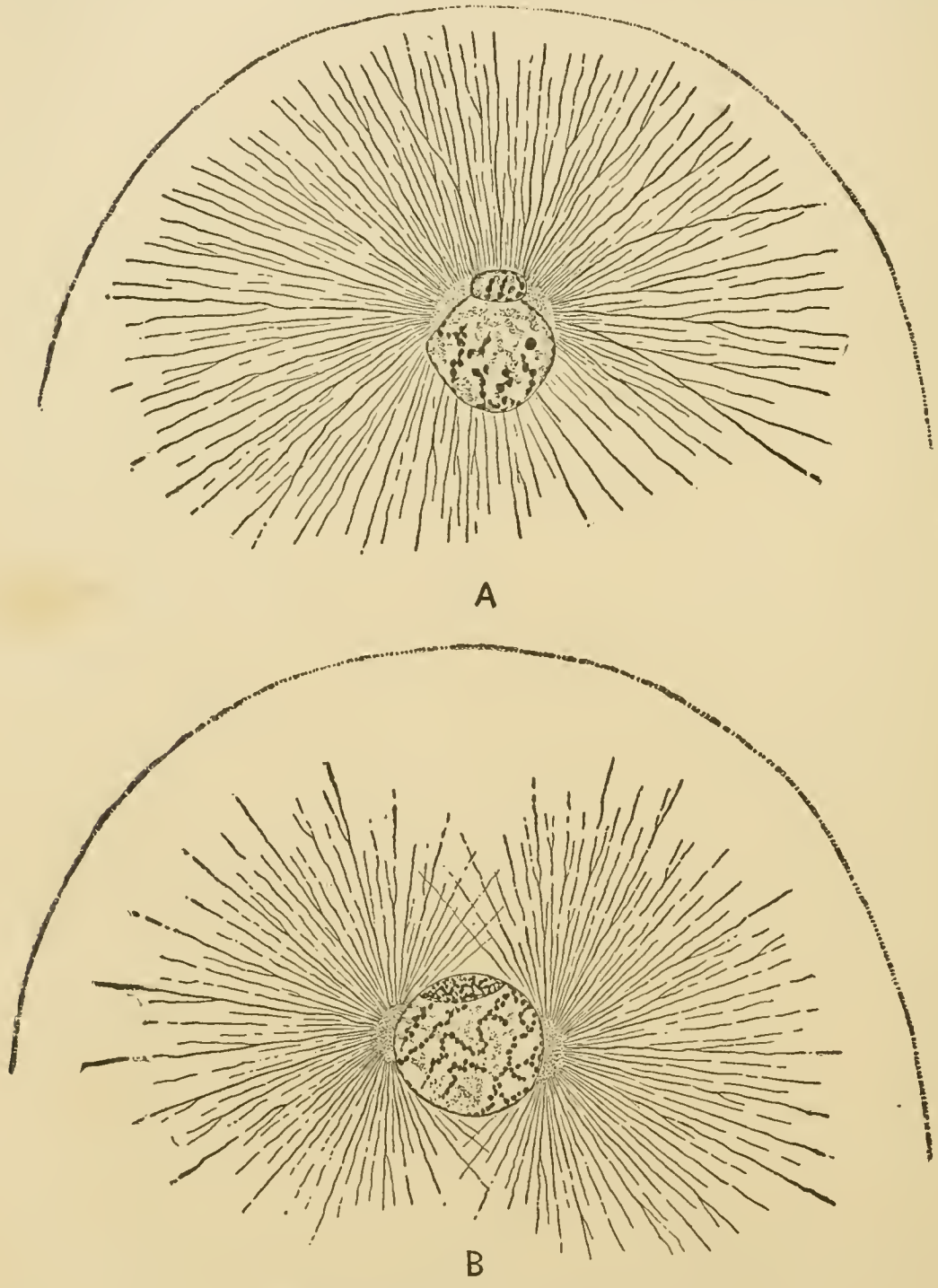


Fig. III. — Conjugation of the nuclei. Extension and division of the aster. [*cf.* Phototype 4.] (10–13 minutes.)

maternal elements remains. In this way a true reticular cleavage-nucleus is formed in accordance with the original description of Hertwig.

During the fusion of the nuclei the cap-shaped central body of the aster draws apart in the middle and finally gives rise to two completely separate halves that place themselves at opposite poles of the cleavage-nucleus. *Pari passu* with this process the astral rays become divided into two systems centred in the respective halves of the central mass. This process does not involve a splitting of the rays, but is caused by their division into two groups through the divergence of their points of attachment. As this takes place, the outer portion of the rays, opposite the equator of the nucleus, are seen to cross one another at an angle, at first very slight, but finally increasing to 90° or even more, — a fact which indicates either that the peripheral portions of the rays must change their position or that new rays are developed in the space between the two half-asters. This crossing of the rays seems very difficult to explain unless they are actual fibres, and not, as Eismond maintains,[1] optical sections of the lamellæ of an alveolar structure. At the close of division each daughter aster has essentially the same structure as that of the original, the rays still reaching nearly to the periphery of the egg.

C. *The "Pause."* — The division of the sperm-aster is followed by a long pause (at a temperature of 27 C., about 15 or 20 minutes) during which remarkable changes occur both in

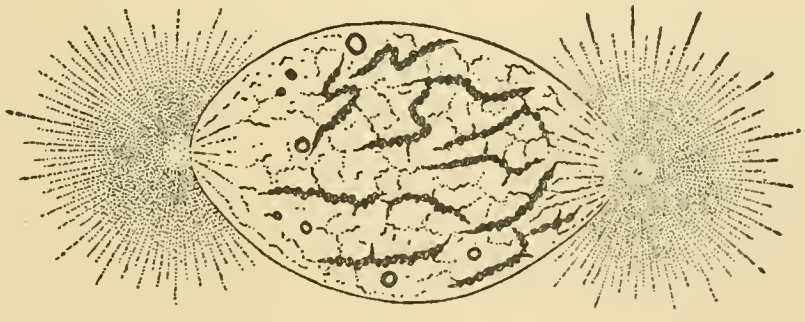


FIG. IV. — Cleavage-nucleus during the "pause," showing the first appearance of the centriole (visible only on one side), the differentiation of the nuclear network, and the beginning of the spindle-formation. [*cf.* Phototypes 5 and 6.] (25 minutes.)

the central mass and in the astral rays. The latter only will be considered at this point.

Immediately after the division of the sperm-aster the astral rays still extend nearly to the periphery of the egg and are

[1] *Anat. Anz.*, X, 7, 1894.

extremely conspicuous. During the pause they become much shorter and less distinct, and in the living egg are nearly invisible, the aster becoming reduced apparently to a clear sphere lying at either pole of the nucleus. Sections show, however, that the rays do not disappear excepting at their outer ends, which either break up into the general reticulum, or are withdrawn towards the basal portion. The aster thus becomes very small, consisting of very thickly set beaded rays extending but a short distance out from the central mass, and staining intensely blue. At this period the rays have lost their stiff and fibrous appearance and plainly consist of regular rows of rounded blue granules arranged in a single series at their bases. The rays are closely crowded, so that the inner portion of the aster often gives the appearance of a homogeneous mass of blue granules,

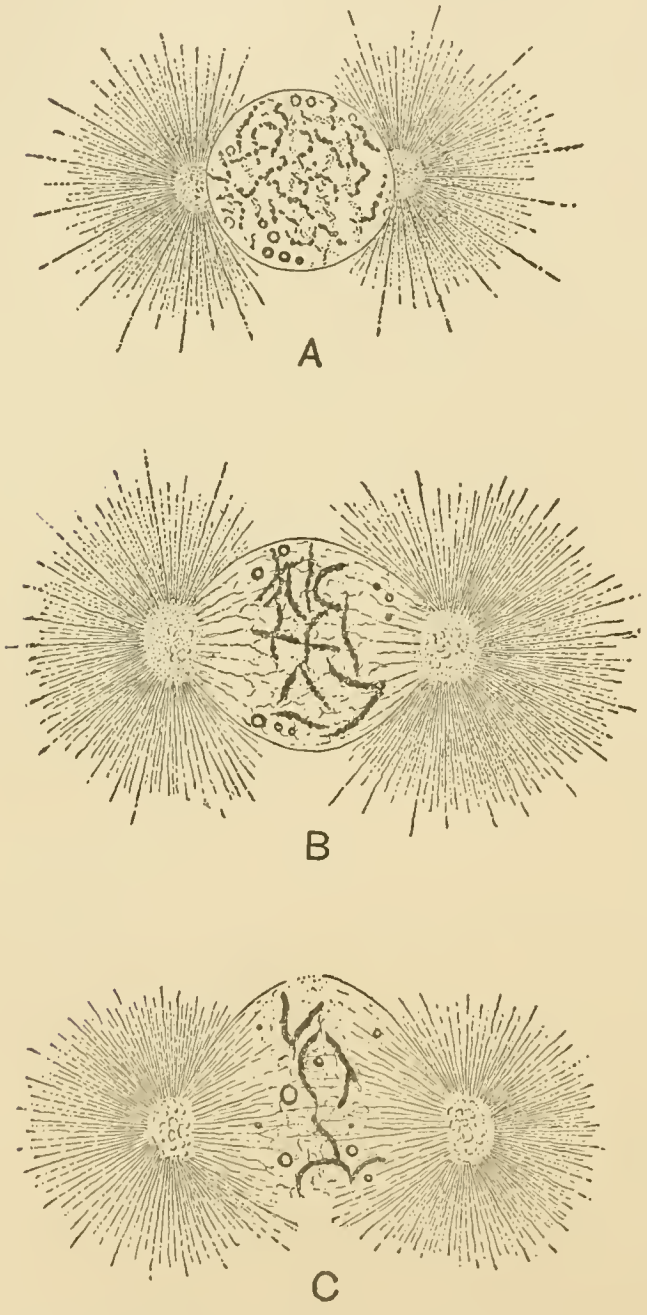


Fig. V. — Successive stages during the "pause." [*cf.* Phototypes 5 and 6.] (25–30 minutes.)

A. After complete fusion of the germ-nuclei to form the cleavage-nucleus. Differentiation of the nuclear reticulum has not begun, but the centrioles have increased in number and form a group of granules in the center of each aster.

B. A stage slightly later than Fig. IV, the chromosomes and spindle-fibres forming, the first indications of a reticulum appearing in the central mass of each aster.

C. Nuclear membrane disappearing, the reticular centrosphere established.

from the periphery of which the rays extend out into the cytoplasm (Phototype 5, Text-fig. IV).

D. *Formation of the Cleavage-Amphiaster.* — The origin of the achromatic figure or cleavage-amphiaster may be followed with the greatest clearness, and shows especially well the formation of the spindle-fibres. As the egg prepares for division the astral rays begin to extend themselves again in all directions from the central mass, still retaining very distinctly their granular structure and never fully resuming the stiff fibrous appearance characteristic of the sperm-aster. Meanwhile the nuclear membrane fades away at the two poles nearest the asters and from the latter spindle-fibres *apparently* grow into the nucleus, and thus give rise to a distinct spindle which lies inside the nuclear membrane (Phototype 6, Text-fig. V).

Further study of the developing spindle-fibres leads to the conclusion that they do not really grow into the nucleus from without, but *are differentiated in situ out of the achromatic nuclear network*. The relation of the spindle-fibres to this network is, in other words, precisely the same as the relation of the astral rays to the cytoplasmic network. The growing spindle-fibres may be seen in the clearest manner to branch out at their ends into the nuclear network, and the latter disappears *pari passu* with the development of the spindle. In some cases the boundary of the spindle exactly coincides with the nuclear membrane, and in this case the membrane-substance also seems to be converted into the spindle-substance. In other cases, however, the membrane may be separated from the spindle by a considerable space, and fades away in this position without relation to the spindle. In the completely formed amphiaster the spindle-fibres are closely similar to the astral rays, but are more closely crowded (so that the spindle appears somewhat bluer) and their granular character is not apparent until a later period.

All these facts point to the conclusion that the achromatic nuclear substance is fundamentally of the same character as the cytoplasmic reticulum, and it will be shown hereafter that in the closing phases of karyokinesis, both astral rays and

spindle-fibres are again resolved into the cytoplasmic network.

E. *Later History of the Spindle and Asters.* — In the fully formed karyokinetic figure (Phototype 7, Text-fig. VI) the spindle appears to be composed of rather stiff, well-defined fibres having a somewhat irregular course, but so closely crowded that it is difficult to determine whether they branch or not. The rod-shaped chromosomes are arranged in a flat plate extending entirely through the equatorial plane of the spindle, and there is no indication whatever of a distinction

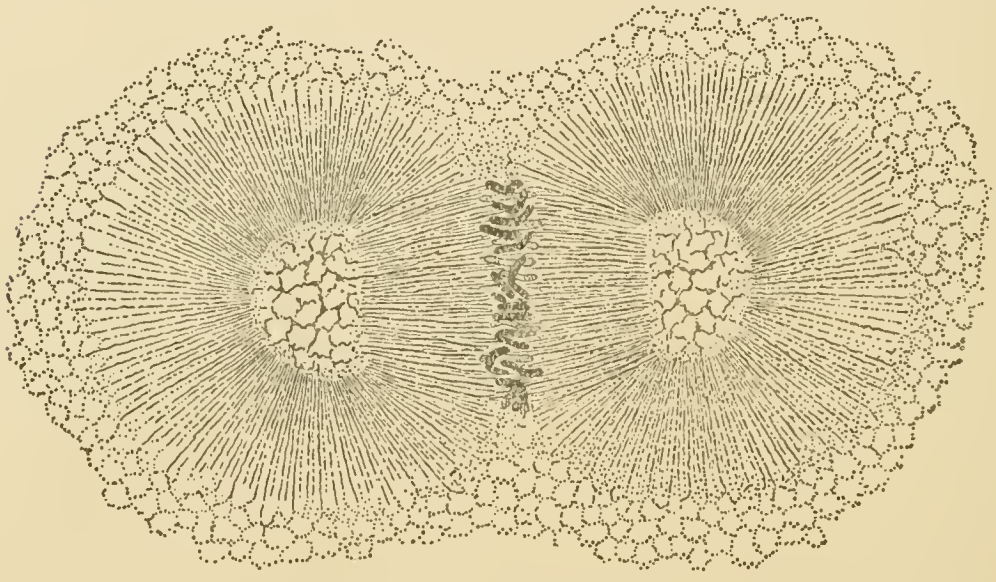


Fig. VI. — The fully formed karyokinetic figure disappearing with a portion of the surrounding reticulum. [*cf.* Phototype 7.] (42 minutes.)

between a "central spindle" and a surrounding layer of "mantle-fibres" such as Hermann has described in the case of Amphibia. As the daughter-chromosomes move apart, the space between them is occupied by conspicuous fibres ("interzonal fibres") which differ from those of the distal portions of the spindle in no visible respect excepting that they are less crowded. Soon, however, their course becomes more wavy, they become granular, and by the time the daughter-chromosomes have reached the spindle poles their central portions break up into separate blue granules, as a rule aggregated to form a vague lenticular mass which appears to represent the mid-body ("Zwischenkörper") of other forms (Phototypes 8, 9, 10, Text-figs. VIII, IX, X). As the cell divides, the remain-

der of the spindle-fibres undergo a similar granular degeneration, the entire spindle breaks up into granules, and in its place appears the usual cyto-reticulum. The study of a large number of preparations, in every stage of the process, leads to the conclusion that the spindle-granules give rise directly to the reticulum, though it is in the nature of the case difficult to exclude the possibility that the granules may be absorbed and the reticulum re-formed.

Immediately after the cleavage, each aster has a horse-shoe shape, and closely corresponds in general appearance to the sperm-aster just before its division (Phototype 11, Text-fig. X, *A*). The subsequent changes are nearly a repetition of those involved in the division of the sperm-aster, each aster dividing into two, while the rays become much shorter (Phototype 12, Text-fig. X, *B*). In the "resting" two-cell stage each blastomere is closely similar to the original ovum during the "pause." The asters persist throughout the two-cell stage, and their descendants may be traced continuously up to the 32-cell stage at least. Beyond this I have not attempted to follow them.

Conclusions regarding the Archoplasm. — I can only interpret the foregoing observations in the sense already indicated at page 446, namely, that the entire system of astral rays and spindle-fibres is the result of a special configuration or rearrangement of a preëxisting reticulum extending through both cytoplasm and nucleus. That this is the true interpretation of the morphological facts observed in the sections, is almost conclusively shown by the fact that *the sperm-aster moves for a considerable distance through the substance of the vitellus.* If with Boveri we assume the astral rays to consist of a specific substance distinct from the reticulum, we must assume the progress of the aster to be a movement of translation through the network, which involves a mechanical problem of extreme difficulty even if we assume the astral rays to end freely and to be of firmer consistency than the reticular meshes. The rays, however, do not end freely, but branch out into the reticulum, and the movement of the aster is hardly conceivable save as a progressive rearrangement of a plastic preëxisting structure under the influence of a center moving through it — an action

in some degree comparable to the progressive rearrangement of iron-filings in a moving magnetic field but differing in the fact that the aster grows as it moves and thus extends its sphere of action (whence the growth of the astral rays).

The term "archoplasm" has accordingly no significance save in a topographical sense; it is that portion of the general thread-work that is for the time being differentiated into astral rays and spindle-fibres, and in view of the misleading etymology of the word, it may well be dropped from the cytological vocabulary as Heidenhain has already suggested.[1]

II. The Chromatin.

The history of the chromatin will be very briefly considered, since my main purpose is to indicate its probable relation to the linin network and to the spindle-fibres. As stated at p. 450 the sperm-nucleus and egg-nucleus completely fuse to form a cleavage-nucleus. During the ensuing "pause" this nucleus greatly increases in size, so that its bulk may finally become four to six times that of the original egg-nucleus (in *Arbacia* as Mathews has shown, the growth at this period is still greater). This growth is not due merely to a swelling of the nucleus, but involves a large increase in the amount of chromatin. Throughout the growth-period the chromatin forms an irregular and discontinuous network, the threads of which consist of rounded granules arranged in irregular strings or cords. Besides these, however, the nucleus almost always contains a number of separate chromatic rings, variable in number and size, which stain precisely like the network. Close examination shows that the chromatin-granules are suspended in the meshes of an achromatic network (linin) the threads of which here and there are free from chromatin and thus come clearly into view. The linin is, however, small in amount and hard to see until a little later.

A. *Prophases.* — At the close of the pause, when the nucleus has attained its maximum size and is preparing for division, its substance undergoes a very rapid and remarkable transforma-

[1] Kern und Protoplasma, p. 154. 1892.

tion which results in the formation of both the chromosomes and the spindle-fibres *within the nuclear membrane*. The linin network suddenly becomes very conspicuous, its substance greatly increasing in amount and staining more deeply, while the chromatin correspondingly decreases, becoming localized to form a number of irregular rods or threads (Phototype 6, Text-figs. IV, V). At first these bodies may be often traced directly into continuity with the less deeply stained linin fibres. Later they become sharply defined and finally break up to form thirty-eight (thirty-six?) chromosomes, which have the form of slightly curved or nearly straight rods which arrange themselves in the equatorial plane of the spindle (Text-fig. VI). *Pari passu* with the formation of the chromosomes the spindle-fibres are differentiated out of the linin network, as already described. It is evident from the foregoing description that there is no proper Knäuel or spirem stage, and I can discover no evidence of splitting until after the chromosomes have been fully formed.

The main point of interest is the sudden increase in the substance of the linin network at the close of the pause. It is possible that this appearance is due simply to a rapid condensation and localization of the chromatic substance whereby the linin network is brought more clearly into view. A careful study of the nucleus at every step of its transformation leads me, however, to regard this as improbable, and I find it difficult to escape the conclusion that a considerable portion of the chromatic portion of the original chromatic substance breaks down into linin, and is thus ultimately transformed into spindle-fibres. This result is less anomalous than it may at first sight appear; for Heidenhain has shown[1] how intimately related chromatin and linin probably are in a chemical sense, and there are a number of well-determined cases in which a considerable portion of the chromatic network degenerates and is converted into "achromatic" nuclear material or into cytoplasm, without entering into the formation of the chromosomes (as for instance in the formation of the polar bodies,

[1] Neue Untersuchungen über die Centralkörper. *Arch. Mik. Anat.*, 43, pp. 542–549.

where in some cases only a fraction of the chromatic substance persists as such).[1]

B. *Later History of the Chromatin.* — The rod-shaped chromosomes arrange themselves in the equatorial plane of the spindle and split lengthwise in the typical manner. Divergence of the daughter-chromosomes always begins at the inner end (towards the axis of the spindle) and proceeds thence outwards, the chromosome assuming meanwhile the form of a Y, the final separation taking place at the outer end, and the daughter-chromosomes of each pair lying end to end. (Text-fig. VII. A photograph will be elsewhere published.) The daughter-chromosomes move rapidly apart and finally pass to

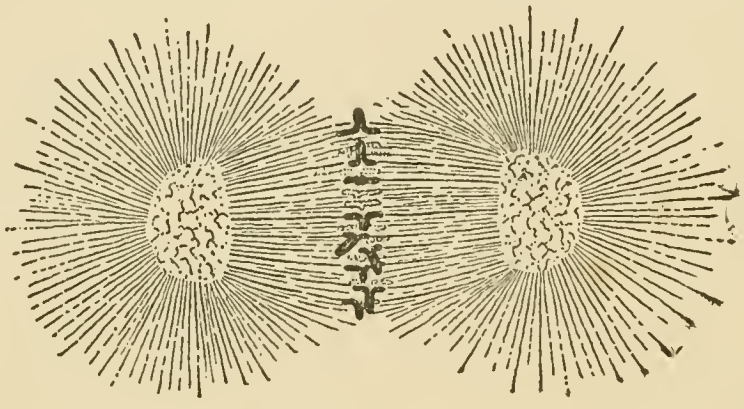


Fig. VII. — Metaphase. Splitting of the chromosomes and divergence of the daughter-halves.

the extreme limit of the spindle, where they lie immediately in contact with the reticular centrosphere (Phototype 8, Text-fig. VIII, *A*). Each of them is now converted into a small spherical vesicle filled with a clear, unstained substance, the chromatic substance forming the wall of the vesicle, and often being aggregated more especially at one side (Phototype 9,

[1] This was shown many years ago by Fol in the case of the echinoderm egg (Recherches sur la Fécondation, etc., *Mém. Soc. Phys. et d'Hist. nat. de Genève*, 1879), and has been observed by a number of observers since. In the starfish *Asterias*, according to Mathews (Journal of Morphology, X, p. 334) by far the larger part of the chromatin is discharged from the germinal vesicle and converted into cytoplasm, an observation I can confirm, from a study of the original preparations, which show that at least nine-tenths of the chromatin is thrown out. The same is true of the acœlous Turbellarian, *Polychœrus caudatus*, according to unpublished observations of Dr. E. G. Gardiner, who has given me an opportunity to examine his preparations, showing the elimination of the chromatin in the clearest manner.

Text-fig. VIII, *B*). The chromatic vesicles then fuse together, their number being progressively diminished until at the time of division there are usually not more than two or three at each side (Phototype 10, Text-fig. IX). As cleavage takes place they finally unite completely to form the small daughter-nuclei (Phototype 11, Text-fig. X, *A*), which are traversed by an exceedingly fine and delicate chromatic network, and stain very slightly. In the ensuing pause or resting-stage the nuclei rapidly grow (Phototype 12, Text-fig. X, *B*). The chromatin resumes its staining capacity, and the succeeding stages closely resemble the history of the cleavage-nucleus.

III. The Centrosome.

The terminology applied to the various portions of the aster is so contradictory and confused that none of the usual terms can safely be employed without clearly defining the exact sense attached to them. This confusion arises mainly from the fact that the word "centrosome" is at present used in three different senses, being applied (1), by Boveri to the entire central mass of the aster exclusive of the rays ("astrosphère" of Fol, "centrosphere" of Strasburger), (2), by Strasburger and others to a smaller dark body often found within the centrosphere, and (3), by Heidenhain to the individual granules of which this dark body is made up. Boveri, than whom certainly no one can speak with a higher right, adopts in his latest paper (*l. c.*) the first of these meanings, and suggests the convenient word "centriole" for the small dark bodies lying within the centrosome of which they form a part. In view, however, of the ambiguity of the word centrosome I shall not employ it in the descriptive part of this paper, but shall adopt Strasburger's term *centrosphere* (equivalent to Boveri's "centrosome" and Fol's "astrosphère") for the spherical reticulated mass occupying the center of the aster in the fully developed karyokinetic figure. The word "centriole" will be applied to the minute deeply staining body ("centrosome" of Strasburger) that in some cases lies in the center of the centrosphere, in some cases precedes its formation. (This body may apparently represent

in some cases the entire centrosome, in other cases only a portion of it.)[1]

A. *Observations.* — A few moments after the entrance of the sperm-head the middle-piece may in favorable preparations be seen as a definite rounded mass lying at the base of the conical sperm-nucleus, though sometimes its boundary is lost in the surrounding protoplasm. I cannot find in this mass anything to be identified with a centriole. It consists of a uniform finely granular or nearly homogeneous substance which, like the middle-piece of the free spermatozoön, stains a clear pale blue in iron-hæmatoxylin, but after double staining with hæmatoxylin and a red plasma stain like Congo red is purplish or reddish. About the middle-piece as a central mass, the astral rays are formed, and their thickened bases are directly continuous with its substance (Text-fig. I).

The central mass rapidly increases in size as the aster moves towards the egg-nucleus, and upon coming in contact with the latter extends around one side of it like a cap. It then draws apart into two rounded masses which place themselves at opposite poles of the cleavage-nucleus, each surrounded by long astral rays which soon afterwards shorten up and become more granular, as described at p. 452.

Up to this period the central mass retains its original structure, and shows no trace of differentiation. Now, however, a change, both morphological and chemical, takes place in its substance. A small, ill-defined mass appears in the center of each aster, in the immediate neighborhood of the nuclear membrane, composed of a substance which stains but slightly in the hæmatoxylin but is colored bright red by Congo red. In the interior of this mass appear one or two extremely minute granules (centrioles), which stain deep blue in the hæmatoxylin (Text-fig. IV). The aster is at this period closely similar to those of *Ascaris* at certain periods, as figured by Boveri, and the red mass with its central group of granules corresponds

[1] In my former paper (Journal of Morphology, X, 1, 1895) I have termed the centrosphere an "attraction-sphere" or "archoplasm-sphere." In view, however, of Boveri's identification of this mass as the centrosome, this terminology becomes so misleading that it will be abandoned in favor of the above.

exactly, I think, to Boveri's "centrosome" with its "Centralkorn" or "centriole" ("centrosphere" with its "centrosome" in Strasburger's terminology). The granules now rapidly increase in number, and thus give rise to a central group lying in the centrosphere which at the same time increases in size. As

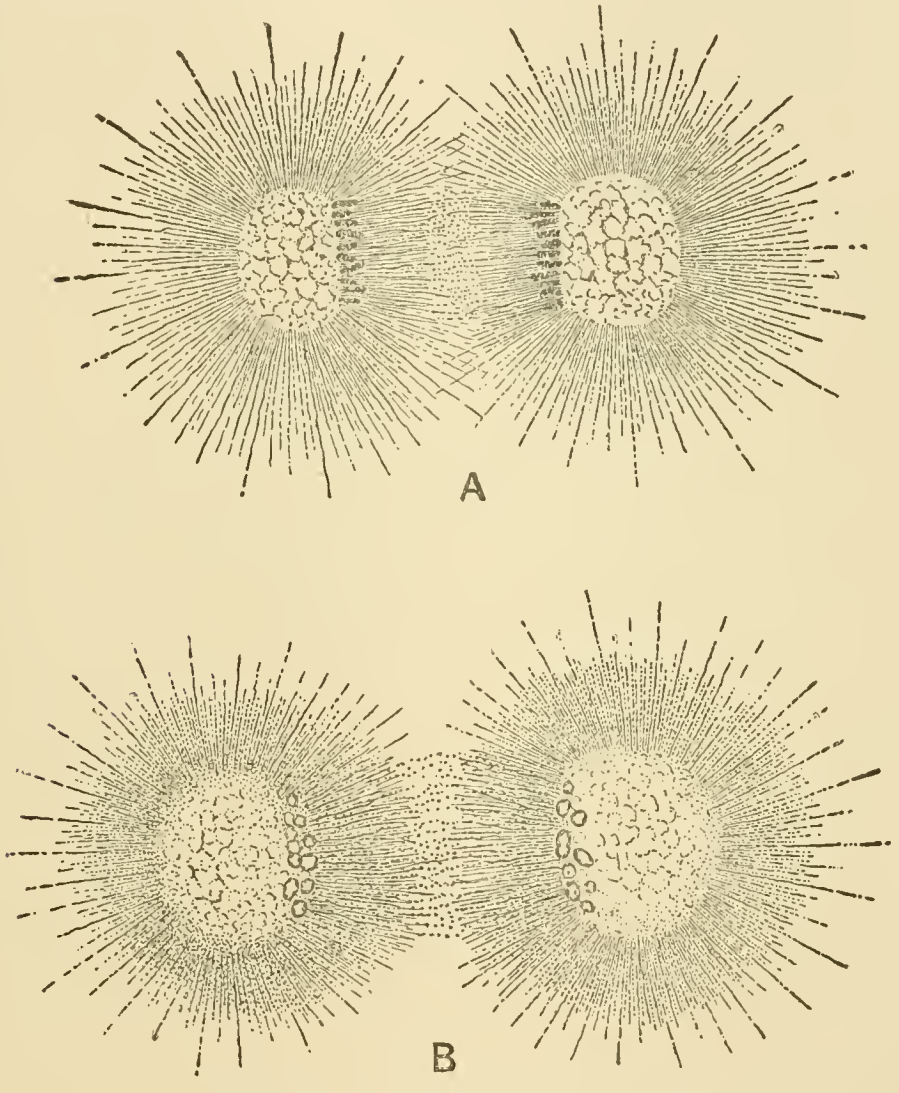


FIG. VIII. — Late anaphases. (50 minutes.)

A. Daughter-chromosomes at the extreme limit of the spindle in contact with the centrosphere. Enlargement of the latter. Degeneration of the spindle-fibres. [*cf.* Phototype 8.]

B. The chromosomal vesicles lying partly in the centrosphere. [*cf.* Phototype 9.]

the granules increase in number they are found to be connected by delicate threads, forming a close reticulum, and with the highest powers the granules seem to be nothing more than nodal points in the network (Text-fig. V). The granules meanwhile change their staining power, taking the hæmatoxylin very feebly and staining red with Bordeaux or Congo red.

In the fully formed amphiaster the center of each aster is occupied by a large, sharply defined, nearly spherical centrosphere, traversed by a rather open granular network. Its substance is now left nearly unstained by hæmatoxylin, but is strongly colored by Congo red, so that, after double staining, its contrast with the blue rays is very marked. During the anaphase the centrosphere becomes still larger and finally attains a truly enormous size, the maximum point being

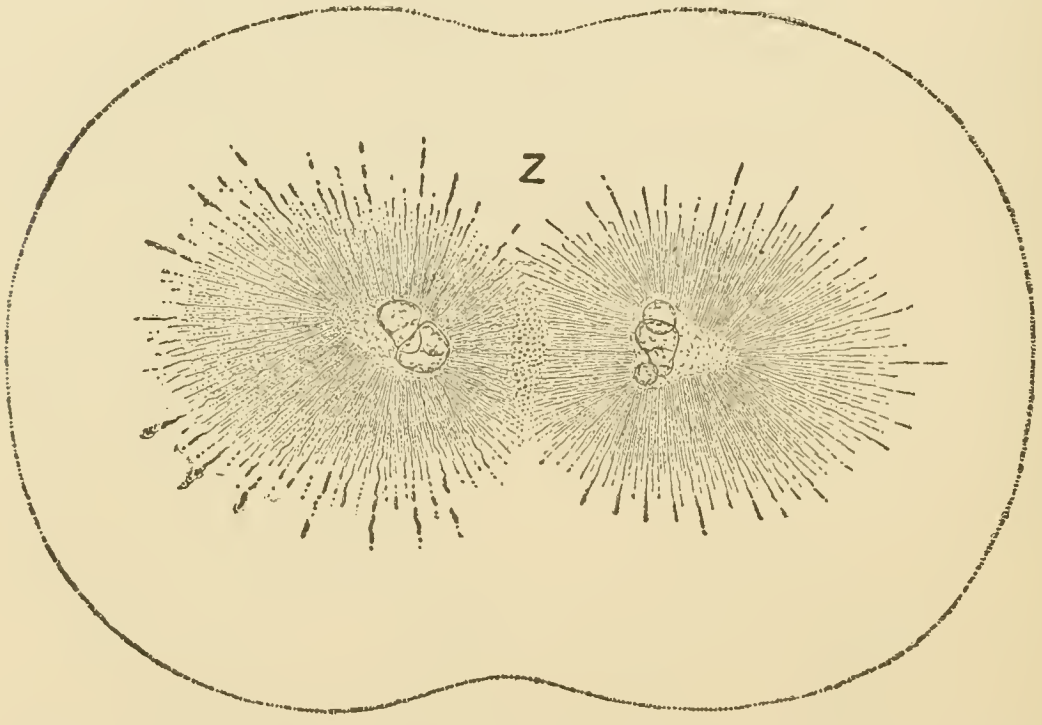


Fig. IX. — Cleavage in progress. Reconstruction of the daughter-nuclei. The chromosomal vesicles have conjugated, leaving only two larger vesicles on one side and three on the other; these now lie in the shrunken remains of the centrosphere. *Z*. Group of granules (degenerated spindle-fibres) forming a transitory "Zwischenkörper" or mid-body. [*cf.* Phototype 10.]

reached at the time the daughter-chromosomes attain the limit of the spindle (Phototype 8). During the reconstruction of the daughter-nuclei the centrosphere again undergoes a remarkable metamorphosis. As the chromosomal vesicles are formed the border of the centrosphere becomes somewhat vague and its substance again assumes the blue-staining capacity, so that the whole body is difficult to see (Phototype 9). As the chromosomal vesicles conjugate, the centrosphere rapidly diminishes in size, its outlines become irregular, it extends more or less completely around the chromatic vesicles,

and, in some cases at any rate, actually surrounds them completely. The egg now divides, and the remains of the centrosphere are now found as a clear substance staining pale blue, that extends more or less completely around the daughter-nucleus (Phototypes 10, 11, Text-figs. IX, X). The entire aster now divides into two, the central mass of each daughter-aster being formed of a nearly homogeneous or finely granular substance, which I believe to be derived from the remains of the centrosphere[1] (Phototype 12, Text-fig. X). The later history of the aster repeats, step by step, the changes undergone by the asters of the "pause," as described at p. 452. Precisely as before, I can at first find no centriole in the central mass. Later a group of minute granules appears, surrounded by a red mass, and by the multiplication of these granules a reticular centrosphere is formed as before.

B. *Conclusions.* — The foregoing observations show conclusively, I think, that the reticular centrosphere (centrosome of Boveri) of the aster arises by a morphological and chemical differentiation of the central mass of the sperm-aster, and that in its earliest stage it is represented by a very minute granule or group of granules forming what would ordinarily be called a "centrosome," but which I have called a *centriole*. Does this centriole have the morphological value of a centrosome? — *i.e.*, is it, in Boveri's words, a distinct permanent cell-organ, that multiplies by division and thus affords the dynamic centers for the formation of the daughter-cells? If my observations are correct, this question must be answered in the negative; for the centriole is not distinguishable, either in the original middle-piece, in the undivided sperm-aster, or in the asters of the early 2-cell stage. I am compelled therefore to conclude that the centriole is formed endogenously in the central mass, and that it is without morphological significance, being only an expression of a secondary differentiation of the central mass caused by unknown chemical and physical activities centering

[1] This account differs slightly from that given in my first paper (JOURNAL OF MORPHOLOGY, X, 1, p. 325), since I had not at that time seen all the later stages, and failed to observe the disappearance of the reticulated structure of the centrosphere.

at that point. This conclusion seems opposed to those of other recent observers, but is closely related to the results of

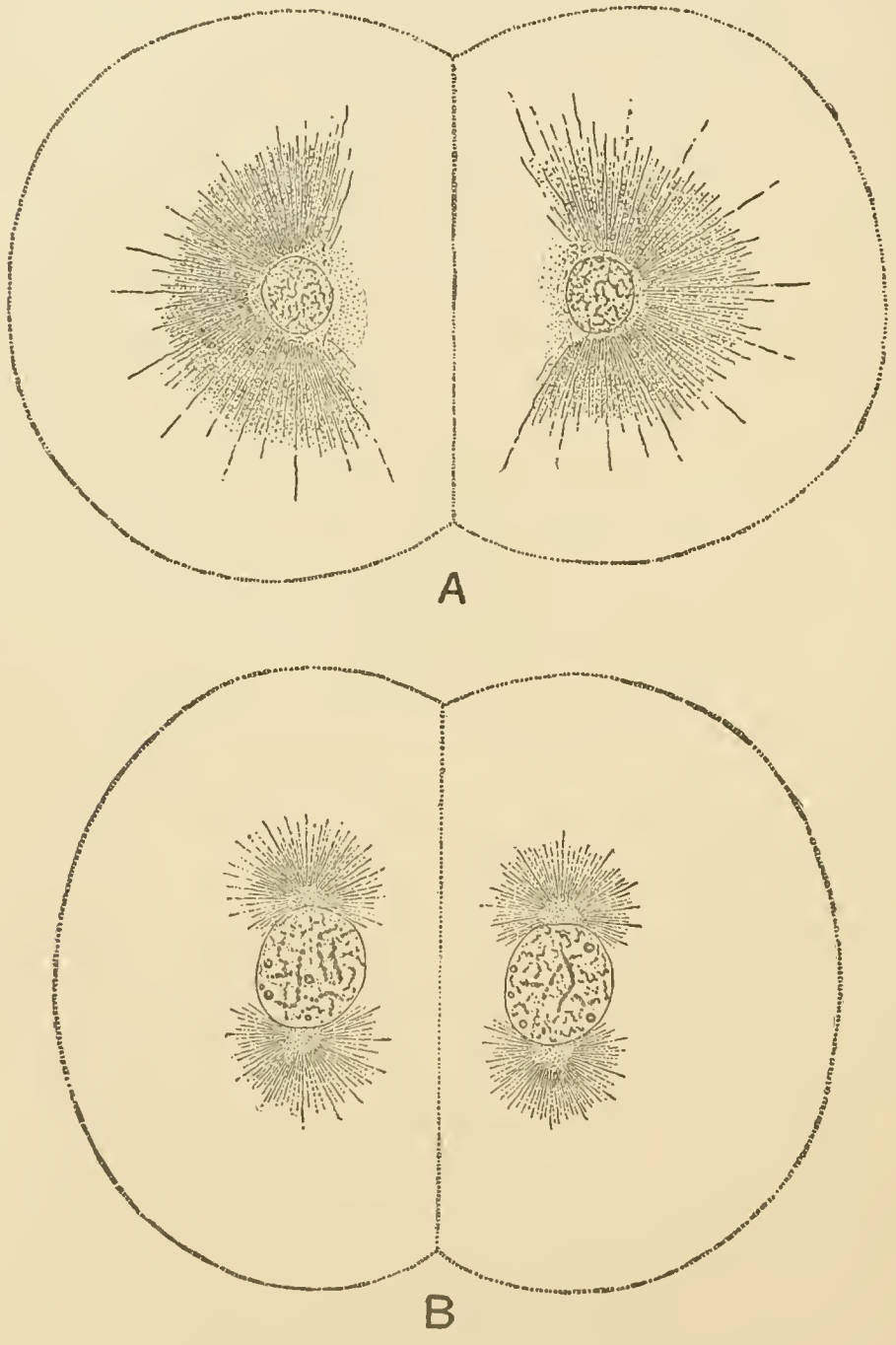


Fig. X.

A. Immediately after cleavage, the asters still undivided. Daughter-nuclei re-formed, surrounded by the remains of the centrosphere. [*cf.* Phototype 11.]

B. Two-cell stage in the pause, the daughter-asters formed but containing no centriole. Growth of the nuclei. [*cf.* Phototype 12.] (60 minutes.)

Vejdovsky's researches on *Rhynchelmis*.[1] Here, likewise, the

[1] Entwick. Untersuch., I. Prag, 1888.

center of the sperm-aster and of each of the daughter-asters derived from it at first consists of a homogeneous mass (*l. c.* p. 84) in which a reticular centrosphere is afterwards differentiated. Within this centrosphere a centriole ("Enkelperiplast") is subsequently formed, arising endogenously through "neue Assimilation des aufgenommenen Materials" as a minute central body (p. 85). This body then divides into two, each of which ultimately enlarges to form a new reticular *centrosphere* (*l. c.*, p. 100) for the succeeding cleavage, and in this a new centriole is again endogenously formed, and so on. In other words, the centriole (centrosome of Strasburger) has here no persistent individual existence, but is formed *de novo* out of the substance of the centrosphere (centrosome of Boveri) at every cell-division, as in *Toxopneustes*. The two forms differ, however, in the fact that in *Toxopneustes* the centriole does not appear until after fission of the aster, whereas in *Rynchelmis* it appears before fission, and leads the way in the fission of the aster by its own division.[1]

What, then, shall we identify in the sperm-aster of *Toxopneustes* as the "centrosome" in Boveri's sense — *i.e.*, as the structure that divides to form the dynamic centers of the ensuing cleavage? I think that the only structure that answers to this definition is the central mass of the aster — that is, the substance of the original middle-piece — without regard to its subsequent morphological differentiation. I cannot deny the possibility that this body *may* contain from the first a centriole (representing the true centrosome) too

[1] It would seem from Boveri's figures and descriptions that the behavior of the centriole in *Ascaris*, is, in some respects, closely similar to what I have described in *Toxopneustes*. In the earlier stages of the archoplasm-masses no centrosome was found (Zellenstudien, II, p. 751, Taf. XX, Figs. 27, 28). It then appears as a small dark body in the center of the archoplasm (p. 752, Fig. 32), divides into two (Fig. 35), followed by division of the archoplasm-sphere, enlarges, and then for the first time a distinct "kleines centrales Korn," or centriole, appears in the center (p. 754, Figs. 36–43). In the later stages of division the centriole is no longer seen (Taf. XXII, Fig. 65–71), nor is it shown in the 2-celled stage *until after the division of the centrosome and archoplasm-sphere* (Figs. 74–77). It would seem, however, that the relation between centriole and centrosome is different in the two cases, though the centrosome is so small in *Ascaris*, and Boveri's references to the centriole so brief, that their precise relation remains in doubt.

minute to be identified, or destroyed by the reagents, or left unstained ; and, in fact, one or more minute blue granules may sometimes be seen near the center of the aster; but the study of a very large number of preparations of apparently faultless clearness and sharpness, leads to the conclusion that these granules are inconstant and indistinguishable from other similar granules lying in the surrounding protoplasm. In *Echinus* Boveri finds *sometimes* ("nur an einigen besonders günstigen Präparaten") a minute deeply staining body in the middle of the sperm-aster, which he identifies with the centrosome, and which he describes as dividing into two just before union of the nuclei. It is impossible at present to determine positively whether this apparent divergence in our results is due to a difference of the methods employed, or to a difference of species. The latter alternative appears to be not improbable, since the arrangement of the astral rays proves that the sperm-aster divides earlier in *Echinus* than in *Toxopneustes* (*cf.* the statements at p. 8 of Boveri's paper with Text-figs. III, *A B* of this paper, which accurately represent the arrangement of the rays).[1] The difference, if it really exist, is, however, of secondary importance, for it relates merely to the morphological configuration of the centrosome, and does not affect the fact that the sperm-aster is developed under the influence of a definite body, derived from the middle-piece of the spermatozoön, which divides into two to form the dynamic centers of the daughter-cells.

IV. On the Mechanism of Karyokinesis.

Van Beneden's beautiful hypothesis of the contractility of the spindle-fibres and astral rays has been almost universally accepted by students of the animal cell, and is supported by such an array of well-determined facts—described by himself and Boveri in the case of *Ascaris*, by Flemming and Hermann in the karyokinesis of amphibian cells, by Solger and Zimmerman in pigment-cells, by Heidenhain in leucocytes — that it is

[1] In the star-fish, as in *Rhynchelmis*, the sperm-aster completely divides before conjugation (Mathews).

hardly possible to doubt its truth. Whether it represents the whole truth is, however, far from certain, and it must be remembered that Strasburger asserts in the most positive manner[1] that the hypothesis is absolutely untenable as an explanation of the movements of the chromosomes in plants.

A study of the facts in *Toxopneustes* shows, I think, that as far as the divergence of the daughter-chromosomes is concerned, it is equally untenable in this case. As shown in Text-fig. VIII, and in Phototype 8, the spindle is barrel-shaped, its truncated ends forming the boundary of the centrosphere at one side, where the spindle-fibres abruptly stop. *The chromosomes proceed to the extreme limit of the spindle* and lie in contact with the centrosphere. This would be impossible were their movements caused by the contraction of fibres stretching between them and the centrosphere. This case shows conclusively, that while the contractility of spindle-fibres may be a true explanation of the chromosomal movements in some cases, yet it cannot be regarded as a universal or the only cause ; and in the present fragmentary state of our knowledge it must remain for future investigation to refer these movements to a common agency.

V. General Summary of Observations.

A. *The Archoplasm.* — 1. The sperm-aster arises by the morphological rearrangement of the general cyto-reticulum under the influence of a central mass derived from the middle-piece of the spermatozoön.

2. The astral rays arise by the linear arrangement and fusion or close union of the granules or microsomes of the reticulum.

3. The spindle-fibres are entirely formed within the nucleus, being progressively differentiated out of the linin network from the centers of the asters, precisely as the astral rays arise from the cyto-reticulum.

4. At the close of karyokinesis the spindle-fibres break up into granules (which may be loosely aggregated to form a tran-

[1] *Anat. Anz.*, VIII, 67, 1893.

sitory mid-body or Zwischenkörper) and are finally resolved into a portion of the cyto-reticulum.

5. The asters persist after cell-division and finally themselves divide to form the daughter-asters which persist throughout the ensuing "resting-stage."

B. *The Chromatin.* — 6. A true reticular cleavage-nucleus is formed by complete fusion of the two germ-nuclei.

7. The amount of chromatin largely increases during the "pause" following the fusion of the nuclei. At the close of the pause a large part of the chromatin appears to be converted into linin, and from this the spindle-fibres are largely derived.

8. The staining power of the chromatin is at a minimum immediately after reconstruction of the daughter-nuclei. It rapidly increases during the pause and reaches a maximum when in the form of chromosomes.

C. *The Centrosome.* — 9. The central mass of the sperm-aster, derived from the middle-piece, forms the centrosome (in Boveri's sense) and at first contains no distinguishable centriole.

10. The centrioles first appear in the daughter-asters derived by the fission of the sperm-aster, and are probably formed by endogenous differentiation.

11. The centrioles represent the first stage in the formation of a large reticulated erythrophilous centrosphere (centrosome of Boveri). Whether this body represents the entire substance of the original central mass (*i.e.*, of the middle-piece) or only a part of it is undetermined.

12. At the close of division the centrosphere greatly decreases in size and finally divides to form the central masses of the daughter-asters, in which the reticular structure is no longer apparent.

13. Centrioles are again formed endogenously in the daughter-asters and the history of the mother-aster is repeated.

VI. General.

The egg of *Toxopneustes* shows with striking clearness the fact, which has been urged by several observers in other cases, that all the parts of the cell show a most intimate morphological connection and may be regarded as specially differentiated areas in a common structural basis. This basis is here a thread-work, the fibres of which are composed of definite granules or microsomes, suspended in a clearer substance of different staining power. While the precise nature of this structural basis in general is still in dispute, it is now pretty generally admitted to form either a network or an alveolar structure that extends throughout the cell, forming outside the nucleus the so-called cyto-reticulum and within it the linin network. According to my observations in *Toxopneustes*, which agree closely with those of Reinke, Eismond, Heidenhain, and many others, the entire system of astral rays and spindle-fibres arises solely through a morphological rearrangement of the elements of this reticulum, as may be observed, step by step, in the formation and later history of the sperm-aster. Moreover, the centrosome itself is but a differentiated portion of the same reticulum, as appears with special clearness in the large centrosphere of the sea-urchin egg, and has been urged on various grounds by Bütschli, Watasé, Reinke, and Eismond.

I can find no sufficient grounds for regarding the nucleus in a different light, and the evidence of its close morphological relationship with other portions of the cell is steadily accumulating. It is true that the chemical composition of the nucleus is highly characteristic (though nuclein is known to exist in cytoplasm as well) and that its morphological independence is very marked, but in neither of these regards is it a unique and isolated structure. Heidenhain has produced evidence that the chromatic network contains substances transitional in a chemical sense between chromatin ("basichromatin" of Heidenhain) and linin ("oxychromatin"); and the nature of the transition is placed in a very clear light by the researches of Kossel, Malfatti and Lilienfeld on the relation between nuclein and nucleo-albumins, the former containing a high percentage

of nucleic acid and having (like chromatin) a special affinity for the basic anilin dyes, the latter containing a lower proportion of nucleic acid and having (like linin) a special affinity for the acid anilins.[1] From a strictly morphological point of view the evidence is growing that chromatin may be directly or indirectly converted into so-called cytoplasmic elements, as in the formation of yolk-nuclei[2] of linin-fibres and spindle-fibres as I have here described; in the discharge of chromatin into cytoplasm occurring in the formation of the polar bodies, or in the periodic elimination of chromatin from the nucleus in the development of *Ascaris* as described by Boveri. Without denying the probability of true intra-cellular symbiosis in the case of the chromatophores we may therefore with Bütschli regard intra-cellular differentiation in general as the result of a particular configuration of a continuous morphological structure.

There is no doubt that the morphological differentiation of parts within the cell is accompanied by corresponding chemical differentiations, and from a physiological point of view these must in the long run be referred to local differences of metabolic activity. This fact is most conspicuous in the case of the nucleus (or chromosomes) and the chromatophores which contain, *and have the power of manufacturing*, specific substances (nuclein, chlorophyll) of comparatively well-determined composition. It is no less certain, as Heidenhain has insisted, that the centrosome possesses a specific chemical composition to which its remarkable effect on the cytoplasm must be due; and both in this case and that of the nucleus the chemical nature of the organ undergoes periodic changes during the cycle of cell-life as shown by regularly recurring changes in staining reaction, such as those that have been described in the present paper. From the physiological point of view, therefore, a "cell-organ" is a differentiated area of the cell-substance in which a specific form of chemical change occurs.

[1] See Lilienfeld, Ueber die Wahlverwandschaft gewisser Zellenelemente zu Farbstoffen. *Arch. Anat. u. Phys., Phys. Abth.*, 1893. Also, Zacharias, Ueber Chromatophilie. *Ber. d. Deutsch. Bot. Ges.*, 1893.

[2] See Calkins on the yolk-nucleus of *Lumbricus*. *Trans. N. Y. Acad. Sci.*, June, 1895, and many earlier papers there cited.

The undetermined question is whether such areas can arise in a morphological sense *de novo*, for example, as focal points in a moving equilibrium of forces pervading the cell as a whole, or whether they are always the effect of a material body pre-existing as a formative center and having a persistent identity, as expressed by the power of growth and division, together with the persistence of the daughter-bodies. Speculative cytology has of late no doubt tended towards the latter conclusion, and, as far as the nucleus considered as a whole is concerned *omnis nucleus e nucleo*, appears to be a statement of universal application. There are, however, facts which raise doubts as to how far this case can be taken as typical of other parts of the cell, and the most striking of these facts are afforded by the history of the nucleus itself. We see here the chromatic substance giving rise to bodies, the chromosomes, which are periodically differentiated in characteristic form and number out of an unformed basis, which grow, divide by fission, and are finally restored again into the common basis, merging into it their own individuality. In this case only the specific material of the dividing body persists, while its morphological individuality disappears; and there is absolutely no proof that the chromosomes emerging from the network at the succeeding division are the same "individuals" (*i.e.*, the same group of individual molecules or other ultra-microscopical units) as before, or that the formative center of each chromosome is a material body. The constancy in their number may with equal reason be regarded as the outcome of formative process (*i.e.*, at bottom chemical changes) affecting the chromatin-mass as a whole and causing it to crystallize, as it were, in a particular form at certain focal points.

May not other cell-organs, not only those that are mere temporary structures, such as pseudopodia or cilia, but also such as are capable of growth and division, arise in a similar manner and be the result rather than the cause of the chemical differentiations that they exhibit? It may be said with justice that this is but a hypothetical restatement of the problem, which explains nothing. But even such a hypothetical suggestion may have some value, if only as a protest against the

dogmatic assumption that each and every localized form of cell-activity must be referred to the agency of corresponding pre-formed material germs. It has, moreover, the advantage of enabling us to conceive the external aspect of the process by which "permanent" cell-organs may have arisen in a historical sense; and it enables us to bring under a common point of view the origin of identical structures either by "free formation" or by the division of a preëxisting parent-structure, as would seem to be the case with the centriole,[1] and is possibly the case with the centrosome as a whole.[2] The uninterrupted continuance of a specific form of metabolic activity at a particular area may be accompanied by the persistence of the cell-organ that is its morphological expression; its cessation may lead to the disappearance of the organ, as occurs in case of the centrosome in echinoderm eggs after formation of the polar bodies; the resumption of the action may create the organ anew. Thus we are led by a study of cell-organization to a point of view not far from that which Sachs has developed on a quite different basis for the organization of the plant body,[3] and from which Loeb has so suggestively considered the development of animals.[4] This view gives in itself, it is true, no immediate insight into the mystery that still envelopes the orderly determinations of differentiations; yet it seems to me full of suggestions for further study.

Biological Laboratory of Columbia College,
July, 1895.

[1] The fact may be recalled that in the Metazoa exactly equivalent structures may be formed either independently or by the fission of a parent structure, as, for instance, the gill-slits of ascidians. (See Willey, Amphioxus and the Ancestry of the Vertebrates, Macmillan, 1894.)

[2] The most careful search has thus far failed to reveal the existence of a centrosome in some tissue-cells, which are nevertheless capable, under appropriate stimulus, of undergoing typical karyokinetic division (as, for instance, the cells of the pancreas and kidney). In the latter case the "free formation" of the centrosome must be regarded as an open possibility, as has been admitted by a number of competent writers. (See Heidenhain, Neue Untersuchungen über die Centralkörper, pp. 654, 655; Watasé, Origin of the Centrosome, *Biological Lectures*, III, p. 287.)

[3] Stoff und Form der Pflanzenorgane, *Gesammelte Abhandlungen*, II, 1893.

[4] On Physiological Morphology, *Woods Holl Biological Lectures*, II, 1893.

DESCRIPTION OF ILLUSTRATIONS.

The accompanying photographic illustrations, enlarged from 950 to 1000 diameters, are from sections of the eggs of *Toxopneustes variegatus*, Ag., fixed in sublimate acetic and stained with iron hæmatoxylin (see p. 444). They are reproduced by the gelatine process, which is entirely mechanical, involving no hand-work or retouching of the plates, and is therefore absolutely faithful to the original negatives. The latter were taken by Dr. Edward Leaming with the Zeiss apochromatic oil-immersion, 2 mm., projection eye-piece 4.

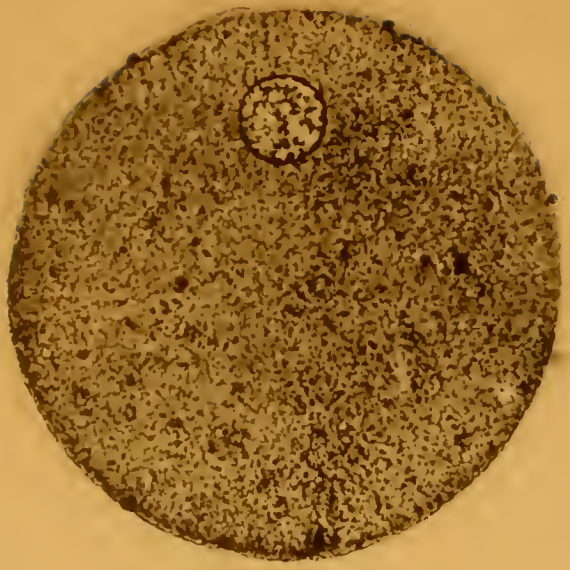
EXPLANATIONS OF PHOTOTYPES 1–4.

PHOTOTYPE 1. The egg a few seconds after entrance of the spermatozoön, showing the egg-nucleus above, the dark lance-shaped sperm-nucleus just inside the lower periphery. A single sperm-nucleus lies outside at the upper right-hand side. The entrance-cone lies at one side, and does not appear (cf. Text-fig. I, *A*, p. 447).

PHOTOTYPE 2. The germ-nuclei approaching (about 5 minutes). Sperm-nucleus rotated about 90°, with small sperm-aster lying at its base (Text-fig. I, *C*, p. 447, is drawn from this specimen).

PHOTOTYPE 3. The germ-nuclei immediately before union (7 minutes), showing growth of the aster. The branching of the astral rays and their microsomal structure is shown at several points. Both the nuclei are slightly out of focus, the object being to show especially the rays (cf. Text-fig. II, p. 449).

PHOTOTYPE 4. Soon after conjugation of the nuclei (10 minutes). From a specimen double-stained with hæmatoxylin and acid fuchsin, which obscures the rays, but shows clearly the central mass of the aster extending like a cap upon one side of the egg-nucleus preparatory to its division. The sperm-nucleus appears as a black body above the irregular egg-nucleus (cf. Text-fig. III, p. 450).



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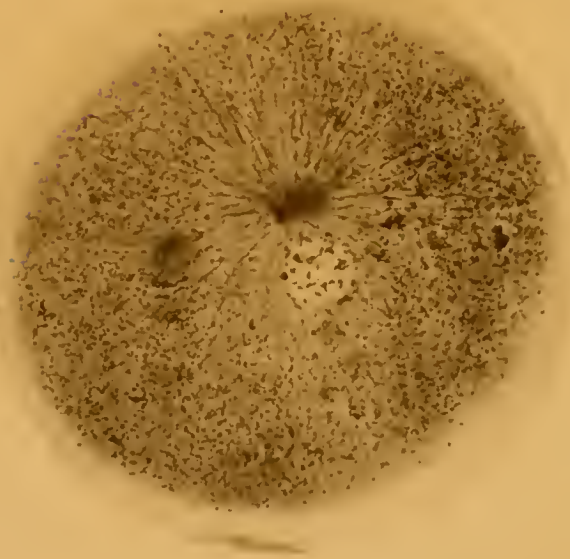

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EXPLANATIONS OF PHOTOTYPES 5–8.

PHOTOTYPE 5. The "pause" (25 minutes), after complete fusion of the germ-nuclei to form a cleavage-nucleus, and division of the aster. The focus is sharply on one of the asters, showing the short beaded rays. In the specimen an extremely minute centriole can be seen in one of the asters, but this does not appear in the photograph.

PHOTOTYPE 6. Late pause (30 minutes), showing initial stage in the formation of the spindle and the differentiation of the nuclear reticulum. A group of centrioles is faintly shown in one of the asters (cf. Text-fig. IV, p. 451, Text-fig. V, p. 452).

PHOTOTYPE 7. The karyokinetic figure just before the splitting of the chromosomes becomes apparent (42 minutes; cf. Text-figs. VI, VII, pp. 454 and 458).

PHOTOTYPE 8. Anaphase (48 minutes), with the daughter-chromosomes lying in contact with the large reticular centrosphere or centrosome. The astral rays can be traced out into the cyto-reticulum, which is clearly shown. The spindle is degenerating (cf. Text-fig. VIII, *A*, p. 461).

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EXPLANATIONS OF PHOTOTYPES 9–12.

PHOTOTYPE 9. The egg immediately before division (50 minutes), showing the chromosomal vesicles, the vagueness of the centrosphere and the beginning of the mid-body (cf. Text-fig. VIII, *B*, p. 461).

PHOTOTYPE 10. The beginning of cleavage (50 minutes). Daughter-nuclei re-forming. The mid-body distinct (Text-fig. IX, p. 462).

PHOTOTYPE 11. Cleavage nearly completed. The daughter-nuclei are re-formed, spindle and mid-body have disappeared, and the asters have assumed a horse-shoe shape preparatory to division (cf. Text-fig. X, *A*, p. 464).

PHOTOTYPE 12. Two-cell stage after division of the asters, in the pause (60 minutes). Growth of the daughter-nuclei. No centrioles can now be seen in the asters (cf. Text-fig. X, *B*, p. 464).

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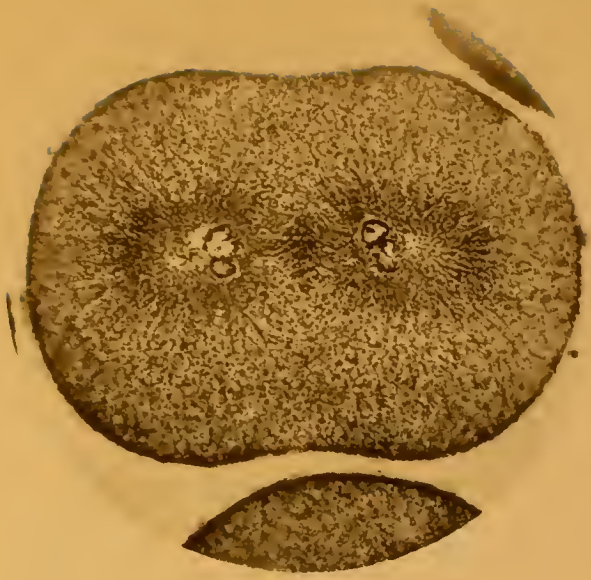

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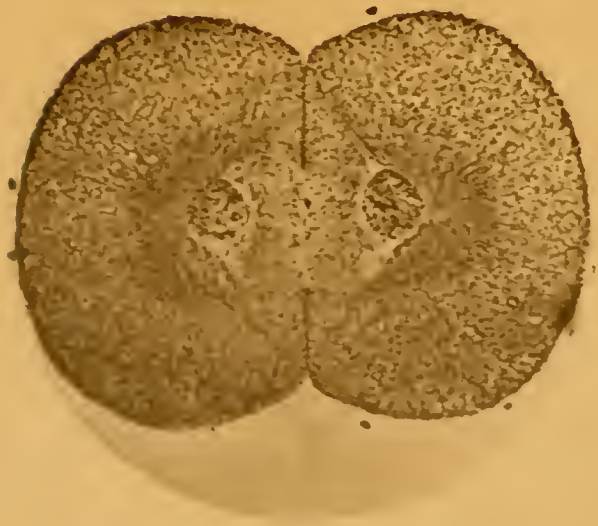

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THE DERIVATION OF THE FRESHWATER AND LAND NEMERTEANS, AND ALLIED QUESTIONS.

THOS. H. MONTGOMERY, Jr., Ph.D.

In a recent paper de Guerne[1] has given a discussion of the origin of the freshwater nemerteans, which, however, is but a brief collaboration of the facts of previous writers; so that I am led to a further consideration of the same problem, and to a more detailed discussion of some of the interesting questions in connection with it.

I have previously attempted a revision[2] of the freshwater nemerteans, — of those at least which have been sufficiently diagnosed, according to which we have four well-defined species belonging to the genera *Monopora*, *Stichostemma*, and *Nemertes*(?)[3] Freshwater forms have been found in Europe, in England, France, Germany, Russia, Switzerland, Austria, and Italy; in Turkestan, at Taschkend; in Africa, Kingani River; and in North and Central America. Five species of land nemerteans have been described, four belonging to the genus *Geonemertes* and one to *Tetrastemma*; the latter was discovered in the Bermudas, the *Geonemertes* species respectively in the Pelew Islands, Australia, New Zealand, and Frankfurt (Germany).[4]

Now when the land and freshwater faunas have been more thoroughly investigated, — for at the present time they are known much less completely than the marine faunas, it is safe to predict that land and freshwater nemerteans will be found

[1] *Ann. and Mag. of Nat. Hist.*, X, 1892.

[2] *Zeitschr. f. wiss. Zool.*, LIX, 1, 1895.

[3] It is not yet determined whether *Nemertes polyhopla*, Schmarda, is a true *Nemertes*.

[4] *Geonemertes chalicophora*, Graff, was found among exotic plants in the Frankfurt botanical gardens and so is probably not indigenous there. The description of *Geonemertes Novae-Zelandiae*, Dendy, appeared in *Annals and Mag. of Nat. Hist.*, December, 1894, after my previous paper had been put in print.

in very many localities, from which they have not yet been reported. But, nevertheless, judging from the fact that these forms are in our present state of knowledge far fewer numerically than the marine forms, and that additions to the latter are constantly being made, it is correct to conclude that by far the greater number of species are marine. Accordingly we have to consider the seas as the original home of the nemerteans, and those forms occurring in freshwater or on land we must regard as marine species which have adapted themselves to new environments.

Freshwater species may have been produced in two ways. In the first place, at a locality where a river empties into the sea, certain individuals of a given marine species would gradually accustom themselves to the less saline water at the mouth of the river, and penetrating by degrees further up the latter, would develop into freshwater forms more or less structurally distinct from the ancestral type. Thus the *Stichostemma eilhardi* of the neighborhood of Berlin, represents probably an originally marine species which has penetrated from the North Sea up the River Spree; and a similar migration up other rivers has probably led to the development of most nemerteans found in rivers. Other forms, however, as has been elucidated by Du Plessis,[1] represent species of a *fauna relicta*, in that an inland sea which was formerly saline, has gradually become freshwater, producing in this way freshwater forms. Such has probably been the origin of the nemerteans in the Swiss lakes, and in similar inland seas. Accordingly, freshwater nemerteans have been produced from marine species, (1) by either an active migration up into bodies of fresh water, or (2) by remaining in the same locality while their environment itself has changed; in other words, we might speak of an *active* or a *passive* adaptation, both of which might lead to the same end.

As to the derivation of the land nemerteans, two possible sources are present: (1) either they are derived from marine forms, or (2) from freshwater forms. On the ground of the first possibility, we must suppose that they had accustomed

[1] Sur l'origine et la repartition des Turbellariés de la Faune profonde du lac Léman. *Actes Soc. Helv.* 60, 1878.

themselves gradually to shallower water, — occurring first below tides, then between tides, and finally above high tide level; or they might have accustomed themselves to salty marshes, which in certain periods of the year become dry, so that the worms would be left on dry land for shorter or longer periods, and then adapt themselves to land which is never covered by water. But though this might possibly be the source of the land nemerteans found near a sea-coast, as the two species occurring respectively on the Pelew Islands and on the Bermudas, we must rather expect that those forms occurring at a greater distance from salt water, and in the neighborhood of rivers and lakes, have descended from freshwater species. That is, individuals of a freshwater species, becoming by degrees accustomed to shallow water and marshes, which are subject to periods of drought, would gradually accommodate themselves to dryer places, until they would be able to live on land which is but slightly moist. That the freshwater origin is the more probable for most if not all land nemerteans, is based on three reasons: (1) if they were descended directly from marine species, we would expect the individuals to increase in number as we approach the sea-coast, which, however, does not seem to be the case; (2) the land nemerteans (as is also the case in the *Turbellaria*) are numerically fewer than the freshwater forms, while if they had developed directly from marine species, they should be at least as numerous if not more abundant than the freshwater forms. And thirdly, we should expect that the land nemerteans are more closely allied to freshwater than to marine forms, because land forms are derivatives of freshwater forms in all those groups of the Invertebrates, which are of marine origin. Thus the closest relatives of the land *Turbellaria* (*Triclads* and *Rhabdocoelcs* [1]) are freshwater species; the same holds good for the land Annelids (*Oligochaeta*), and for the land pulmonate *Mollusca*. Accordingly I feel justified in concluding, that as the freshwater nemerteans are derived from marine species, so the land nemerteans are derived from freshwater forms; there is, of course, the possibility that a small number of the land nemer-

[1] Only one land Rhabdocoele is known, *Prorhynchus sphyrocephalus*, v. Graff.

teans have developed directly from marine species, though there is no substantial proof for this view.

Now it should be an aim of investigators who are comparing marine with freshwater and land faunas, to determine from which marine species the others have been derived, and what structural modifications have been evolved by the change of environment. Such an investigation could be best pursued at a locality where a river empties into the ocean; and by determining what species occur on the neighboring sea-coast, at the mouth of the river, and further up the river, it would be possible to deduce the different degrees of adaptation to the new environment, and the resulting stages of structural modification. In the case of the nemerteans, such a study could be carried on in Germany at the river Spree, or here in the United States of America in Delaware Bay and the Schuylkill river,—since in both localities we find marine species at the mouth, and freshwater species further up the river. As to structural differences caused by the change of environment, the fact occurs to me that, while the nearest marine relatives of the freshwater forms (with the exclusion of *Nemertes polyhopla*, Schmarda) are forms with four eyes (genus *Tetrastemma*), the majority if not all the freshwater forms possess a greater number of eyes (from four to eight). Thus the change from salt to freshwater would seem to necessitate a larger number of eyes, due perhaps to the different conditions of light; and it would be interesting to determine whether the same law holds good in allied groups, —as the *Turbellaria*. There is a fact of importance in this connection, namely, that hand in hand with an increase in the number of eyes of the freshwater nemerteans, takes place an increase also in the variability of their number. While among about a hundred individuals of the marine *Tetrastemma vermiculum* which I examined alive at Newport and Woods Holl,[1] the number of eyes was invariably four; and among about fifty individuals of *Tetrastemma candidum* studied at Woods Holl, in only one case I found five eyes; in the freshwater *Stichostemma eilhardi*, on the contrary, I found no constancy in their

[1] I wish to express my thanks to the U. S. Fish Commission, for the use of a room at their laboratory at Woods Holl, where this article was written.

number, — only a few individuals possessed four eyes, while the majority showed five, six, seven, or eight. Now I consider this variability in the number of the eyes of the freshwater forms to be explained by the general law, that all organs (and *propter hoc* all organisms) which are undergoing progressive or regressive development, tend to be variable. There is a progressive development taking place in the eyes of the freshwater nemerteans, with regard to their number, and hence the number is variable, since the action of the progressive development is *still continuing*. Now if this law, which of late years seems to have been lost sight of by most zoölogists, — the law that progressive or regressive development is always accompanied by variability, can be shown to be of general application, and I know of no case to the contrary; then the deduction must follow, that *the amount of variability above or below a given mean will stand in inverse ratio to the length of time in which the development* (*progressive or regressive*) *has acted upon the given organ*. Thus through a great lapse of time the agency of the development producing four eyes in the marine genus *Tetrastemma* has acted, so that a variability in the number of the eyes almost never occurs; on the other hand, the developing agency tending to produce more than four eyes in the freshwater nemerteans, has acted for a comparatively much shorter time, and accordingly the variability in the number of the eyes of these forms is very great. And from all this, it may be possible, by comparing the relative amount of variability in the number of the eyes of marine and freshwater forms, to deduce the comparative age of the latter.

The freshwater nemerteans accordingly differ in the number and variability of their eyes from the allied marine species, and this also seems to be the case with the land forms; but data are wanting in regard to the structural modifications produced by the change of environment in the other organs. Naturally the food obtained in the freshwater would differ from that in the sea, — thus the two marine species of *Tetrastemma* examined by me seem to feed mainly on *Algae-spores* and *Infusoria*, while the freshwater *Stichostemma eilhardi* contain almost exclusively small Crustacea. This difference in the food obtained

might produce also a difference in the structure of the intestine, and a different manner of procuring it might modify the proboscis. Further, the action of different climatic influences, especially the rapid cooling of the bodies of freshwater during the winter, and the periodical cessation of active life caused by this agency, would influence the reproductive periods of the nemerteans, and perhaps also the development. What is needed is a careful study of these questions; and I would feel amply repaid if this little paper would stimulate others to the active study of the interesting problems involved in the change from salt to fresh water, — to an investigation of the modifications of structure, and the agencies which have produced them. And the results of such a study would naturally be of far more use in the attempt to solve the problem of the inheritance of acquired characters, than to experiment upon individuals kept in confinement, by gradually decreasing the salinity of the water. For the objection is justified, that in such experiments the organism is placed in pathological conditions, and that the experimentor is attempting to produce structural changes in a very brief time, which are (perhaps always) produced by natural agencies very gradually, and through long periods of time.

Woods Holl, Aug. 5, 1895.

THE CRANIAL MUSCLES AND CRANIAL AND FIRST SPINAL NERVES IN AMIA CALVA.

EDWARD PHELPS ALLIS.

For several years past I have been engaged in an anatomical investigation of the cranial nerves in Amia calva. The manuscript descriptive of the larger part of the work has been practically ready for publication for two years or more. The publication of it has, however, been delayed awaiting the completion of the numerous drawings necessary for illustrations. These drawings and the final dissections from which they are being made are being prepared by Mr. Jujiro Nomura, my exceedingly able and valuable assistant. I now find, unexpectedly, that several of the dissections and drawings already finished are wanting in definiteness and completeness, and must accordingly be remade, necessitating a further delay of several months. I therefore publish now the following short summary of certain of the results obtained.

1. The canalis transversus of selachians (Gegenbaur) is, in Amia, a groove extending transversely from orbit to orbit immediately in front of the transverse cartilaginous "Wulst" (Sagemehl), and the two ossifications of that "Wulst" that represent in Amia the basisphenoid of teleosts (Bridge).

2. The "Augenmuskelkanal" of Amia and teleosts is not therefore the canalis transversus of selachians, as Sagemehl concludes it to be. In Amia it is a cavity or space formed in late larval stages around the hypophysis and saccus vasculosus; and entrance to it, for the muscles of the eye, has been acquired along a canal that gives passage to two branches of that portion of the orbital venous sinus that lies in the upper lateral chamber of the eye-muscle canal. Of these two branches, one arises in the choroid gland and is almost directly continuous with the other, which arises from the hypophysis and connects under that organ with the corresponding vein of the opposite side of the head.

3. The hypophysis and the saccus vasculosus in the adult of Amia are both glandular structures. Both receive a considerable nervous supply direct from the base of the infundibulum, and both communicate directly with the infundibular cavity.

4. The olfactory nerve, contrary to Sagemehl's statement, lies exposed to the orbit through a limited part of its course. The opening through which it is so exposed lies at the extreme front and upper end of the orbit, and gives passage to a vein coming from the nasal pit. The olfactory canal in front of the opening is therefore formed by the fusion of two canals, the olfactory canal proper and what is probably the orbito-nasal canal of selachians. The opening into the orbit can therefore be called the orbito-nasal opening or fenestra.

5. The "hitherto undescribed cranial nerve" of Pinkus in Protopterus is found in Amia, part of its fibres arising with the olfactorius and part of them having the intercranial course described by Pinkus, though their definite origin from the brain was not satisfactorily determined. In the nerve the large cells described by Pinkus are found, scattered along the nerve in old larvæ, but in 12 mm. larvæ gathered into a knob-like protuberance on the under surface of the nervus olfactorius at about the middle of its length. In general histological appearance this collection of cells on the olfactorius resembles at this age the ciliary ganglion found on the nervus oculomotorius in much the same relation to that nerve. As the ciliary ganglion is unquestionably in part at least a sympathetic ganglion, the ganglion on the olfactorius is probably of a similar character, and is possibly the sphenopalatinum of higher vertebrates.

6. The profundus ganglion is found both in larvæ and in the adult as a separate and distinct ganglion, connected with the ciliary ganglion by a radix longa and with the brain by a profundus root wholly separate and distinct from the root of the trigeminus in larvæ, but somewhat fused with that root in the adult.

7. There is in Amia no true ramus ophthalmicus profundus, a nerve which, when found, lies ventral to the superior branch of the oculomotorius and ventral to the nervus trochlearis.

There is, however, a large and important portio ophthalmici profundi (van Wijhe), single or double, lying dorsal to all the muscles and nerves of the eyeball, and corresponding therefore to those frontal branches of the ophthalmicus profundus that have a similar relation to those nerves and muscles, such as branch 1 of Ewart in Læmargus.

8. The ramus ophthalmicus superficialis trigemini, in Amia, also lies dorsal to all the nerves and muscles of the eyeball. It cannot therefore be sometimes found in other fishes or in Amphibia fused with the ramus ophthalmicus profundus, as is generally assumed to be the case, unless in such cases the superior branch of the oculomotorius and the nervus trochlearis are found as apparent branches of the compound-nerve so formed. Where such is not the case, and where the ramus ophthalmicus trigemini has the relations of a profundus to the nerves of the muscles of the eyeball, as in selachians and Amphibia, the ramus superficialis, if it exists, must be represented by so-called frontal branches given off by the ramus ophthalmicus before it passes under the rectus superior muscle.

9. The ramus palatinus facialis in Amia arises entirely from that part of the trigemino-facial ganglion that is formed on the fasciculus communis root, and its distribution to regions where terminal buds abound is such that it must be concerned in the innervation of those organs, although branches of the nerve could not be definitely traced to any of them. In Rana, Strong states definitely that it innervates certain of those organs.

10. The ramus ophthalmicus superficialis trigemini, in Amia, also arises mainly, if not entirely, from that part of the trigemino-facial ganglion that is formed on the fasciculus communis root, and it is concerned in Amia largely, if not entirely, in the innervation of terminal buds. That part of it that is so concerned should, therefore, be considered either as a branch of the palatinus facialis or as homodynamous with that nerve. As there are no terminal buds, or but few, on the top of the head and snout in selachians and Amphibia (Merkel), the nerve is naturally wanting or small in those forms.

11. The rami maxillaris superior and maxillaris inferior trigemini each receive an important part of their fibres from the

ganglion of the fasciculus communis root, and branches of each are distributed to regions where terminal buds abound: such branches often taking what are apparently circuitous courses to reach regions more naturally supplied by branches of other nerves. Certain branches of the two trigeminal nerves, therefore, probably innervate terminal buds, and such branches probably arise from the fasciculus communis component of the nerve to which they belong. One of these branches, a branch of the maxillaris inferior, becomes the mandibularis internus trigemini distributed to the inner surface of the mandible. It is, therefore, probably the homologue of the palatinus inferior facialis of certain fishes (Protopterus), and hence (Pinkus) the homologue of the chorda tympani of higher vertebrates. As the mandibularis internus facialis is a postspiracular nerve, it cannot be the homologue (Ewart, Strong, Pollard) of the chorda tympani, as that nerve is prespiracular.

12. The pharyngeal and pretrematic branches of the glossopharyngeus are in Amia distributed to regions where terminal buds abound, and branches of the pretrematic nerve were with reasonable certainty traced to certain of those organs. The glossopharyngeus, in Amia, receives no recurrent or communicating branch from the palatinus facialis as it does in Protopterus (Pinkus). The fasciculus communis component of the nerve, if there be such a component, must, therefore, be of intracerebral origin as it is in Rana (Strong) and in birds (Brandis), the funiculus solitarius in Aves being the homologue of the fasciculus communis in Pisces (Strong).

13. The vagus in Rana receives fibres from the fasciculus communis tract (Strong), and in Aves from the funiculus solitarius (Brandis). As terminal buds are found in Amia in the branchial chamber where branches of the vagus are distributed, it may be assumed with reasonable certainty that the vagus in Amia receives fibres from the fasciculus communis tract, and that these fibres innervate the buds innervated by that nerve.

14. Terminal buds represent a condition or stage through which the canal organs of the lateral lines have passed in their development (Wiedersheim). Terminal buds and the nerves

innervating them should therefore arise from or in connection with sensory ectodermal thickenings as do the canal organs and their nerves. Certain pharyngeal or pretrematic nerves are known to innervate certain terminal buds, and the pharyngeal and pretrematic branches of all the cranial nerves arise from or in connection with epibranchial or pretrematic ectodermal thickenings (Beard, von Kupffer). The terminal buds should therefore arise from or in connection with those same thickenings, and as the lens of the eye belongs to the line of these thickenings (von Kupffer, Platt), it may be a modified terminal bud or buds, and one of the ciliary nerves the nerve innervating it.

15. The muscles rotating the eyeball in the several orders of the Ichthyopsida are not homologous structures, if existing descriptions of their innervation can be relied upon. On the contrary, the group Ichthyopsida (the Pharyngobranchii excluded) can be divided by the innervation of the muscles of the eye into two great groups and other sub-groups, which, if reversions have not taken place, indicate distinct and definite lines of descent.

In one of the two great groups, represented by the Cyclostomata alone, the nervus abducens innervates two recti muscles, the inferior and the externus. In the other group that nerve innervates but one rectus muscle, the externus, but it innervates also a retractor bulbi when that muscle is found.

The second group is subdivided into two sub-groups, in one of which the superior branch of the oculomotorius, lying dorsal to the ophthalmicus profundus, innervates two recti-muscles, the superior and the internus, while in the other it innervates but one, the superior. In the first sub-group are found Elasmobranchii, Dipnoi and Urodela: in the second Ganoidei, Teleostei, and Anura. The Amphibia are thus separated into two sub-groups, corresponding to the two sub-groups of Pisces. In the prototype of this second group there must have been an arrangement of muscles and nerves most nearly represented by that found in the Holocephala, where the rectus internus arises near the front end of the orbit, as in Petromyzon, and the

obliquus superior from the edge of the orbit, as in Petromyzon. From this prototype two lines lead to the two sub-groups of Pisces and two to the two sub-groups of Amphibia.

Between the two lines leading to Amphibia lies Ichthyophis, in which the muscles rotating the eyeball are innervated as in Anura, but in which a retractor tentaculi has been formed from one of the muscles of the eye (Sarasins), probably from the rectus internus of Urodela and not from the retractor bulbi, as the Sarasins suggest. Ichthyophis seems therefore, in the arrangement of the muscles of the eyeball, to represent the beginning of the line leading to higher vertebrates, as Burckhardt states that it does in the arrangement of the parts of the brain.

16. The levator arcus palatini and the dilatator operculi together of Amia are the homologues of the muscle called by Vetter in selachians *Add*γ, and their innervation indicates that they are derived from the dorsal half of the superficial constrictor of the mandibular arch, and that they correspond to the levator muscles of the branchial arches.

17. The four muscles called by McMurrich the second, third, fourth, and fifth division of the levator arcus palatini, are not probably parts of that muscle. The second and third muscles are derived from the levator maxillae superioris, and probably also from one of the spiracle muscles, of selachians, while the fourth and fifth divisions are derived from the muscle called by Vetter *Add*β. In teleosts these four muscles become partly absorbed by the adductor mandibulae, and represent the apparently aberrant bundles or insertions of that muscle, such as the muscle $A_3\beta$ of Esox (Vetter), the adductor tentaculi of Amiurus (McMurrich), and tendon A_2^t of Perca (Vetter).

18. The hyomandibular and the symplectic in the hyoid arch, and the metapterygoid process and the anterior process of the metapterygoid in the mandibular arch have, in Amia, practically the same relations to the nerves, muscles, and arteries of those arches that the suprapharyngobranchials and infrapharyngobranchials have to the nerves, muscles, and arteries of their arches. The hyomandibular and the metapterygoid process of the metapterygoid therefore probably correspond

to the suprapharyngobranchials of the branchial arches, and the symplectic and the anterior process of the metapterygoid to the infrapharyngobranchials.

19. The interhyal, or stylohyal, is certainly the epal element of its arch, or epihyal, and the quadrate probably the corresponding element in its arch.

PRELIMINARY NOTE ON THE LIFE-HISTORY OF GONIONEMUS.

LOUIS MURBACH.

THE occurrence of large numbers of a striking and beautiful medusa at Woods Holl, during July and August, presented a favorable opportunity for studying the life-history of a form, only the adult stage of which is known. When it was found to be Gonionemus, discovered by A. Agassiz, in 1862, in the Gulf of Georgia, Washington, and, as far as I can find, not further described since then, I determined to follow out its development.[1]

It seems desirable to give a preliminary account of results up to this time, as the completion of the work may have to be put off until next year on account of the present scarcity of the medusae.

Agassiz's[2] diagnosis is as follows: Gonionemus, A. Agassiz. "The spherosome is an oblate spheroid, cut from pole to pole; the ovaries are in lobes alternating on the sides of the chymiferous tubes, and extending their whole length, from the digestive cavity to the circular tube; the digestive cavity is long and very flexible; the tentacles are numerous, large, and exceedingly contractile; chymiferous tubes are four in number." "Found in July, Gulf of Georgia, W. T., 1862."

In his species description he brings out other features for recognizing the medusae. Such are the violet color of the lobed ovaries, a spot of the same color near the tip of each tentacle, and the fact that the tentacles are kneed at the end (serving to fasten the animal to sea-weed by means of

[1] For working facilities at the Fish Commission Laboratory during this summer, I am greatly under obligation to the Commissioner of Fish and Fisheries. Also to Dr. C. O. Whitman, the Director of the Marine Biological Laboratory, for use of library, *etc.*

[2] Illustrated Catalogue of the Museum of Comp. Zoöl. Harvard Coll., No. 11, North Am. Acalaphae.

their nettling organs). He gives the actinal diameter as $\frac{8}{10}$ of an inch.

The medusae remain rather quiet during the day unless disturbed, but become very active as darkness sets in.

Largest specimens taken were 3 cm. in diameter, and had 64 tentacles; the smallest were 6 mm. in diameter, and had 32 tentacles.

Each tentacle is bent near the end on account of a pad of cement cells on the oral side, by means of which the animal fastens itself to foreign objects. It does not use its nettling organs for this purpose, as Agassiz supposed.

The color varies from deep brown or orange to paler shades, except the chymiferous tubes, which are always dark. The tips of the tentacle frequently are a deep pink.

These medusae are said to have occurred here last year in small numbers in the Eel Pond, a small body of water containing about five acres, with only one outlet into the Harbor. This year as many as two hundred were taken in one evening with the tow-net. They were so much sought after as specimens that it is now difficult to find enough for completing the work.

When seen from the aboral side in the water they are at once conspicuous by the cross formed by their gonads, by the bright green pigment-spot at the base of the tentacles, and by the radiating brown tentacles.

The females are usually darker than the males. This may be due to an abundance of ripe eggs on the periphery of the ovarian folds. The pigment to which the general color of the gonads is due lies in the deeper portion of the gonads.

Eggs and sperm are dehisced from the outside of the gonads by the breaking up of the epithelium.

Dehiscence occurs normally about one hour after twilight — about 8 o'clock at this season of the year. Repeated experiments show that it is dependent on the withdrawal of light, since medusae taken at any time of the day, when placed in the dark for about an hour, deposited sperm and eggs.[1]

[1] Brooks (in "The Life-History of Hydromedusae," *Mem. of the Boston Soc. of Nat. History*, vol. III, No. 12) is inclined to think that marine animals deposit

This discovery made it very convenient for obtaining different stages of segmentation during day-time.

The eggs are spherical granular bodies .08 of a mm. in diameter, and of light brown color. The nucleus is large and usually lies near the periphery of the freshly deposited egg. No egg-membrane could be demonstrated in such eggs until some time after fertilization. As polar bodies were seen on only a few occasions, it is probable that they had formed very early and passed off rapidly. They are minute, and cling together.

The spermatozoa are conical bodies 3 microns long, and 1½ microns broad, slightly constricted near the posterior end, to which a very delicate tail 70 to 100 microns long is attached. In the constricted portion of the body four deeply stainable bodies may be seen from the end to which the tail is attached.

Segmentation begins about 1½ hours after deposition of the eggs. It is total and equal — especially in the earlier stages. The first four segmentation planes divide the egg into 2, 4, 8, and 16 cells, at intervals of about 45 minutes. From this time on, segmentation is not so regular. The cells become columnar, and are arranged around a small cavity giving rise to a blastula. This becomes ciliated and rotates in the egg-membrane, in some cases with watch hands, and in others, in an opposite direction. The blastula pushes out one pole somewhat, and, working its way out of the egg-membrane, it becomes a pear-shaped, free-swimming planula, in from 12 to 20 hours from the beginning of segmentation.[1] The larger end goes foremost. It may persist in this stage for several days or for only 40 hours. Then it elongates, loses its cilia, and attaches by the end corresponding to the larger end of the planula. There now has been a differentiation into ectoderm and endoderm, the latter being formed by multipolar budding of cells in the segmentation cavity. Nettling organs are formed

their eggs at definite hours of the day. He cites four medusae that deposit eggs regularly at 8 P.M. It would be interesting to learn whether any besides Gonionemus can be induced by artificial darkness to deposit during the day-time.

[1] Under certain conditions the ciliated free-swimming stage was entirely omitted, but as this abbreviated development is not yet understood, I defer an account of it until later.

in the ectoderm, and migrate toward the free end of the larva — corresponding with the pointed end of the planula. Usually two opposite tentacles bud out next, though many cases have been observed where only one appeared, and others where two tentacles appeared at right angles to each other.

Thus we have arrived at a hydrula stage, and, so far as this goes, we have true alternation of generations.

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MORPHOLOGY.

CONTRIBUTION TO THE STRUCTURE AND DEVELOPMENT OF THE VERTEBRATE HEAD.

WILLIAM A. LOCY.

CONTENTS.

GENERAL INTRODUCTION.

A GROWING interest has been manifested in the problems of cranial morphology as their importance in comparative anatomy has been more fully realized, and a number of strong investigators have taken up this particular field of study. Through their researches much has been accomplished; the views on cranial anatomy have gradually changed, as advance after advance has been made, until morphologists have come to look upon that wonderful complex — the head — not as a structure *sui generis*, but as the most extensively modified part of the animal, formed by differentiation and specialization from parts that are structurally homologous with those that follow in the trunk. According to this conception, the distinction between head region and trunk region is one of degree of differentiation and not one of kind.

The modifications which the head has undergone have been brought about gradually, and are so comprehensive in their range that if we could know their complete history, even in one animal, we should have a key to the leading questions of vertebrate phylogeny. But there are so many causes tending to modify the course of development, that we cannot depend on the steps of phylogenetic history being repeated in a complete and orderly way in any one animal form. Often a balance of probabilities must be struck to determine what is ancestral and what is secondarily acquired. The chain of evidence is indeed very incomplete, and must always be supplemented by a certain amount of inference ; but what has not been fully enough recognized in the practical study of embryological development, is that the shortest intervals of time may be very important in keeping the connection. Coherency of the history must be preserved ; and the difficulty of doing so is greatly increased by the fact that the new is made to proceed out of the old, and, frequently, one organ insidiously takes the place of an earlier formed one. Too great stress cannot be laid on the desirability of having a more complete series for study, and this is especially important in cranial anatomy. The traditional method has been to study one stage and then another "a little older," and to fill in the intervening gap with inferences. This has proved to be inadequate and misleading. It is now required that we shall have stages close enough together to trace the history of the transitory as well as the permanent organs. The practical difficulties in obtaining a sufficiently complete series are very great, and, in many cases, well-nigh impossible. Great effort has been expended in getting the material for the present research ; it is a kind of material in which the stages cannot be controlled, and my series cannot be regarded in any sense as a complete one. Nevertheless there is represented in it several distinct stages that have not heretofore been described by students of elasmobranch embryology. Beard, in his study on the Transient Ganglion Cells and their nerves in Raja batis says : "My series of the embryos of this form is what many might judge to be complete, numbering as it does some 300

specimens of all ages and sizes. Nevertheless there are gaps in the collection and these are often of a kind that it may not be easy to fill in. No two embryos are exactly alike in all the pictures which they yield of this apparatus, and in half a dozen specimens that would be taken to be of the same age from their sizes, from a comparison with Balfour's stages, or from the more certain criteria of number of gill-clefts, protovertebræ, etc., etc., it is quite common to find this transient nervous system, like other organs, in widely different stages of development and presenting great variations in detailed characters. In my researches on Raja I have been compelled to give up completely any attempt to make use of Balfour's nomenclature. With a limited number of embryos at one's disposal, it is easy to fit them into one or the other of the well-known stages; with an increased number this becomes more difficult or even impossible, so great are the variations met with." I have had a similar experience with my embryos of Squalus acanthias (*Acanthias vulgaris*). I am conscious there are gaps in the material, and, after making the best use of it that I can, I have, no doubt, missed many things not represented in my collection.

The studies recorded in this paper deal almost exclusively with the early history of the brain and sense-organs. Some of the questions upon which direct evidence is brought are: What was the primitive condition of the nervous system of the Vertebrates? What was the number and nature of the primitive neural segments entering into the brain? What has been, in general, the line of modification along which they have been converted into the brain? And what were the early steps in the differentiation of sense-organs?

I have been greatly indebted to Professor C. O. Whitman for courtesies both at Woods Holl Biological Laboratory and at the University of Chicago, and I have also to thank him for advice and suggestions on the subject-matter of this paper.[1]

[1] Soon after the appearance of the Zieglers' paper (Beiträge zur Entwicklungsgeschichte von Torpedo: *Archiv für Mik. Anat.*, Bd. 39, Hft. 1, Jan. 1892) Dr. Whitman suggested to me that I should work over the same ground — the gastrulation and formation of the germinal layers — in one of our North American

In glancing over the views regarding cranial anatomy that have been held since the beginning of this century, we find a suggestive hint in the shift of opinion, as to the decidedly relative nature of all our views on morphological problems. There has been a distinct rise in the point of view, but scarcely any of the earlier problems are yet solved ; new ones have been added, but those that have been handed down have grown broader and increased in proportions as a mountain on nearer approach. There has been a gradual change in front, keeping pace with the advance of our knowledge and with the changes in our methods of interpretation. During that period the very soul has been breathed into the body of comparative morphology, and as a result, our interpretations are directed towards explaining anatomical structures in connection with their past development.

At the beginning of this century, on account of superficial appearances, the conception took rise that the head is divided into definite segments. The obvious division of the skull by sutures was gradually worked into the theory that the cranium represents a number of modified vertebræ; and then began on the part of anatomists, the efforts to determine the number of these segments or vertebræ in the skull. Oken and Goethe formulated the theory which was taken up by that eminent anatomist Richard Owen, who lavished upon it an amount of attention and labor that was worthy of a more substantial theory, but the mistakes of that great man have proved of benefit to other morphologists.

This was the beginning of the idea that the head region represents a definite number of segments. Originally founded on external features of no segmental importance whatsoever, and not essential to the question which they served to introduce, the problem gradually widened and deepened and reached the essential parts connected with this segmental condition. The first conspicuous change of front came with the delivering of

species of Elasmobranchii. I collected material (Galeus canis and Squalus acanthias) for that purpose, and began studies along that line, but, finding new and undescribed conditions in the head region, my attention was gradually drawn off from the original purpose and directed towards cranial anatomy.

the Croonian Lectures, in 1869, by Huxley, in which he completely overthrew the vertebral theory of the skull, withdrawing attention from the superficial sutures in the cranium and directing it to the cranial nerves and branchiæ as bearing evidence to the segmentation of the head. Gegenbaur, in 1872, studied the cranial nerves especially in relation to the branchial clefts and reached the conclusion that there are nine segments represented in the head. Another distinct advance was made by Balfour, who first studied the segmental divisions of the mesoblast in the head of the Elasmobranchs, and identified by this means eight head-somites clearly represented. He also expressed the conviction that there were primitively a larger number of segments but, owing to extreme modifications of the head region, they are no longer clearly represented. This was at bottom the same problem, but it was now shifted upon organs that are truly segmental.

From the time of its discovery, this segmental division of the mesoblast in the head became a great favorite with morphologists in elucidating the problem of head segmentation. The mesoblastic divisions seemed, so far as the evidence went, to embody the most direct survivals of the original segmentation and, therefore, to be the most promising line along which to work out the problem. Valuable contributions have been made along this line since Balfour's time, by Marshall, Van Wijhe, Dohrn, Killian, Oppel, and others, and the myotomes of the head have continued to hold their high position in the minds of morphologists as the most significant remnants of the original segmentation.

Notwithstanding all these researches the original problem is still unsolved. There is no agreement as to the number or the nature of the primitive segments, and about the only point that may be regarded as settled beyond controversy is that the head and brain were primitively divided into segments.

Recently, there has been added as a factor in the discussion, observations on segmental divisions of the neural tube. Although such divisions have attracted the attention of several observers, their importance in the problem of cranial segmentation has not been appreciated. They have been regarded as

of secondary origin, depending on the segmentation of the mesoblast. Nevertheless, I hope to show that these segments are the first to appear in the head region, and that they are entitled to more serious attention on the part of anatomists, as representing the most primitive segmentation of the head of which any traces are preserved.

PART I. — METAMERISM OF THE HEAD.

I. Basis for the Discussion.

The more recent discussions on the metamerism of the head are based upon segmental divisions as shown in (1) cranial nerves and branchial clefts, (2) mesoblastic head cavities and (3) segments of the neural tube.

The first-mentioned basis may now be set aside as involving too much conjecture. McClure has stated the objections to it as follows : "We have positive proof that the degeneration of certain branches has taken place. This being the case, we have every reason to assume that whole segmental nerves may have once existed, which have completely degenerated, leaving no trace whatever of their previous existence. If such be the case, the segments originally connected with these degenerated nerves must necessarily be overlooked, if the existing nerves are made use of as a means of determining the original number of segments.

"Furthermore, the vagrant changes in the position of some of the cranial nerves must necessarily cause confusion. For example, take the sixth nerve, which in the frog and tadpole stages is situated between the first and second roots of the ninth nerve, a position somewhat posterior to its place of origin. This remarkable shifting clearly shows not only what great changes in position the cranial nerves are capable of undergoing, but it also goes to prove that we can find no reliable means of determining the primitive segments by means of their connection with the exit of the existing cranial nerves. Beard in taking up this problem made use of an important series of sense-organs for which he has proposed the name of 'Branchial Sense Organs,' from their development from thickenings

of the epiblast over each branchial cleft. The dorsal branches of certain cranial nerves fuse with these epiblastic thickenings; the superficial part of the thickening giving rise to a branchial sense-organ, while the deeper portion becomes the ganglion of the dorsal root of the cranial nerve. This close relation which exists between the dorsal branches of the cranial nerves and their corresponding sense-organs is undoubtedly of segmental character. But this line of research is beset by a great difficulty, namely, that the degeneration of certain branchial sense-organs would, in time, involve the degeneration of their corresponding cranial nerves, and such degeneration has certainly taken place, in part or in whole, leaving in doubt the primitive segments with which they were connected."

The second and third points mentioned are more important clews to the metamerism of the head. Muscle and nerve are, physiologically, so fundamentally related that we should naturally expect some close correspondence between muscle segments and neural segments, and metamerism of the head region should be studied in light of the work done on both sets of structures.

The myotomes (or muscle segments) have received by far the most attention as they are the more conspicuous, but it is timely to ask whether they afford the most reliable evidence as to the primitive number of brain segments. Comparative study shows that the neural segments are the first to appear and are less subject to modifications than the muscle segments of the head. The large number of myotomes described in the head of selachian embryos by Dohrn and Killian are more transitory than the neural segments. The period in which they are exhibited is a short one, and soon the seventeen or eighteen segments of Killian, and the eighteen or nineteen of Dohrn, become reduced, by fusion, or absorption, or both, to the nine head segments of Van Wijhe.

The neural segments, on the other hand, begin very early, as shown in this paper, and preserve their original number and characteristics through several embryonic periods. It will be seen as we proceed in the account of these segments, that the assumption cannot be sustained, that the segmental divisions of the middle germ-layer (protovertebræ) are primitive.

II. Historical Review of the Work on Neuromeres.

The question of Metamerism of the Head as based upon myotomes has been completely reviewed by Dohrn, Killian, and others ; I shall say nothing on that side of the problem, but shall limit the historical review, and confine the discussion to the side of the question that has been less cultivated.

It is a fact of comparatively recent discovery that the whole neural tube of vertebrates is divided by constrictions into similar segments. Each segment is bounded, anteriorly and posteriorly, by transverse folds ; and the elevated area between them constitutes the segment to which the name metamere is given. These segments may be pictured to the mind as a series of transverse ridges and furrows occupying each side of the neural tube and not extending across the median plane. They are exhibited in very young embryos of Vertebrates and disappear before what may be called the middle embryonic period. The existence of such folds in the walls of the hind-brain has been known since the time of Von Baer, who in 1828, first observed them in the embryonic chick of the third day of development ; but it was not until 1889 that they were known to extend throughout the length of the neural tube.

Since Von Baer's time they have been observed and commented upon by various anatomists. Bischoff [1] figures the neural segments, but does not mention them either in the text or in the descriptions of the figures. His figures show seven folds in the region of the fourth ventricle of a dog embryo of the twenty-fifth day of development. There are also shown three additional folds in the region of the mid-brain.

Remak, in 1850, made important observations, and suggested that the segments in the hind-brain are connected with the origin of the nerves in that region. He noted five or six quadrilateral fields on each side of the hind-brain walls, calling attention to the fact that they correspond closely in position

[1] I am greatly indebted to Hoffmann's historical review of the literature in Bronn's Klassen und Ordnungen des Thierreichs. I have consulted nearly all the literature referred to there, but, in some few cases, where the original papers have been inaccessible, I have depended wholly upon his review of it.

with the visceral arches, and with the cranial nerves "which grow with them." According to his observations they fade away after the fifteenth day.

Dursy observed them in 1869, in the embryonic cow, of 6.5 mm. in length. He recorded the occurrence of six folds in the region of the fourth ventricle. Foster and Balfour, in 1874, noted the same structures in the chick, and suggested that they were of segmental importance. Dohrn, in 1875, called attention to the occurrence of eight or nine neural segments in the fourth ventricle of bony fishes. He contrasted this early segmentation with segmental divisions in insects. Götte figures such segments in the hind-brain of well-developed embryos of Bominator. In 1877, Mihalkovics was inclined to interpret these segments as due to mechanical pressure of the mesoblast, and, therefore, not a fundamental feature of the medullary tube.

Béraneck showed, in 1884, that there is a definite connection between certain of these segments and cranial nerves, thus giving the first real foundation for establishing their segmental relations. In his earlier paper, he describes five pairs of transverse folds in the hind-brain of embryos of Lacerta agilis from 3 to 4 mm. in length. He noticed that they rapidly fade away and disappear in embryos 5 or 6 mm. in length. In 1887, he studied the relations of these "replis médullaires" in the chick, and, as regards their connection with cranial nerves, reached similar conclusions.

Kupffer maintained in 1885 that these segments indicate a primary metamerism of the medullary tube. He has published several brief notices on these structures. In 1884, he gave a record of studies on the brain of the trout, in which he found five pairs of neural segments in the hind-brain. In sagittal sections he noted, in addition to these, three pairs in the mid-brain. He found no segments further forwards, and concluded that the fore-brain is not to be included in the segmented region.

In the following year ('85) he gave the results of his studies on embryos of Salamandra atra. In embryos of that form, showing as yet no traces of protovertebræ, he found

eight pairs of neural segments in the median part of the hind-brain. It is to be carefully noted that these segments observed by Kupffer were in embryos with a wide open neural groove, and occupied the median part of the cephalic plate, thus giving a "mediane Gliederung des Hirnes." In 1893, in his "Vergleichende Entwicklungsgeschichte des Kopfes der Kranioten," he gives figures (20*a* and 20*b*) of the forms described in 1885. These figures of Salamandra atra show segmental folds only in the median part of the neural plate, and none in the neural ridges. This is interesting when compared with my observations on amphibian eggs (see p. 529).

In addition to the eight segments in the brain region he counted thirteen or fourteen in the cord, extending backwards to a point a little in front of the blastopore.

Rabl ('85) speaks of unmistakable segmentation in the hind-brain of chick embryos of from fifty to ninety hours' incubation. He found seven or eight segments in the region of the fourth ventricle — not being able to determine definitely whether there were seven or eight. Again, in 1892, Rabl has most ably discussed the question of the metamerism of the head, but as his paper deals almost exclusively with segmentation in the mesoblast, it does not come in for attention in the present connection.

Oscar Hertwig gives the matter passing attention in the third ('88) edition of "Lehrbuch der Entwicklungsgeschichte." He is not inclined to attach much importance to the neural segments.

Gegenbaur, also, does not look upon these particular segments as important factors in the metamerism of the head. His position on the question is shown by the following quotation so frequently met with: "So interessant und so vielversprechend diese Thatsachen sind, so wenig scheinen sie mir gegenwärtig geeignet, zur Beurtheilung der Metamerie des Kopfes selbst als Factoren in Geltung gebracht zu werden."

Orr, in 1887, traced very definitely the connection between these segments in the hind-brain and cranial nerves. In describing the segments he made use of the term "neuromeres," which has been generally adopted on this side of the Atlantic.

He describes six in the hind-brain of the lizard (Anolis), giving their anatomical characteristics with great clearness. He observed no neuromeres behind the point of origin of the tenth nerve, nor did he find them in the fore- and mid-brain, but he concluded, hypothetically, that they were present in the anterior brain regions. Orr found the fifth, seventh and eighth, ninth, and tenth nerves, respectively, connected with the first, third, fifth, and sixth neuromeres of the hind-brain.

Hoffmann, in Bronn's "Klassen und Ordnungen des Thierreichs" (1888), records his observations on these segments in Lacerta and Tropidonotus. He found seven in the hind-brain of these forms. In the following year he added further details in the *Zoologischer Anzeiger*. He differs somewhat from Orr as regards the relationships of the cranial nerves, assigning the fifth nerve to the second neuromere of the hind-brain, the seventh and eighth to the fourth, the ninth nerve to the sixth, and the tenth nerve to the seventh neuromere. From the first segment the fourth nerve arises, and subsequently shifts its position forwards.

McClure, following Orr's work, demonstrated the segmentation of the neural tube throughout its whole extent, and published a preliminary announcement of the same in 1889. He showed the presence in the spinal cord of segments continuous with those in the brain, and histologically similar to them. He examined these structures in the chicken, Amblystoma, and the lizard (Anolis). He fixed upon six in the chicken and lizard, and five in Amblystoma, as the number in the hind-brain of each respectively. He found two in the fore-brain, but left the number in the mid-brain undetermined, expressing the view, however, that there are two neuromeres in that brain region. Thus he identifies ten neuromeres in the entire brain region, and agrees with Orr in the assignment of nerves to the neuromeres of the hind-brain.

Miss Platt's work (1889), on "Axial Segmentation of the Chicken," agrees, in so far as neuromeric segmentation is concerned, with that of her predecessors, except as regards the relation of the nerve-fibres to their corresponding neuromeres. According to her observations they spring, primarily, from the

concavity between two segments, and not from the crest of a neuromere. My observations on the motor roots agree with those of Miss Platt in that particular.

Zimmerman ('91) states that he finds in embryos of the rabbit and chick, shortly before the closure of the neural groove, the segments observed by Kupffer in Salamandra atra. He noticed at first eight of these segments (encephalomeres) in the brain region. The three anterior ones were much larger than the five lying behind them in the medulla. The three front ones are the vesicles of the fore-, mid-, and hind-brains, and they straightway undergo secondary division as follows: The first divides into two, the second into three, and the third into three, making a total of eight secondary divisions arising from three primary ones. These added to the five of the medulla give a total of thirteen segments in the brain region. He also observed these structures in Acanthias and Mustelus, and found them very clearly defined. In mammals the metameres of the mid-brain are not so distinct. Zimmerman goes on to say that these folds cannot be accidental appearances, since in all classes of vertebrates corresponding nerves arise from corresponding segments. He gives a table, showing nerve relations, with too much detail to reproduce here.

Waters, whose complete paper appeared in June, 1892, studied especially the mid-brain of Teleosts. He confirmed and extended the observations of Orr and McClure. He counted eleven neuromeres in the entire brain region: six in the hind-brain, two in the mid-brain, and three in the fore-brain. He did not find neuromeres in the brain of the Cod earlier than the ninth day of development. He assigns the olfactory and optic nerves to the anterior two neuromeres. The two neuromeres of the mid-brain give origin to the third and fourth nerves, and from the six segments in the hind-brain the fifth, seventh, eighth, ninth, and tenth nerves arise as designated by McClure. The sixth nerve he found to occupy its theoretical position when the neuromere exists; when fusion has taken place between the trigeminus and abducens neuromeres the sixth nerve has been shifted backwards between the seventh and eighth nerves.

Froriep gave a noteworthy contribution to the subject of neuromeric segmentation in very early stages, before the Anatomische Gesellschaft of Germany, at the June meeting in 1892. He described anew the so-called neuromeres that he had previously observed in mole embryos, but concluded that they are not of true morphogenetic significance. He further described the conditions in Triton embryos, and concluded that the so-called primary neuromeres detected by Kupffer in those animals are simply the result of underlying mesoblastic somites.

Froriep agrees with Kupffer in finding segmental folds while the neural groove is widely open, and in locating them in the median part of the cephalic plate. But, whereas Kupffer finds eight in the brain region of Salamandra atra, he finds only four in the corresponding region of Salamandra maculosa, and five in Triton cristatus. (Compare with my observations, p. 529.) His general conclusion is that "the jointing of the vertebrate body is originally determined by the middle germ-layer; when ectodermal structures exhibit segmental arrangement, it is the result of secondary adaptation."

Herrick ('92), in a preliminary paper, gives an account of neuromeres in the Ophidian embryo, in stages after the complete closure of the neural groove, and after the formation of the ear vesicle. His figures show six neuromeres in the medulla. He states a proposition that will be of use later, in helping to distinguish between primary metamerism, and metameres of secondary origin which show after the closure of the neural tube: "If neuromeres once existed in the fore-brain they would be visible only at an early stage . . . The so-called fore-brain neuromeres differ from those of the medulla and cord in involving only dorsal structures."

The present writer, in 1894, gave the first account connecting the earliest formed neuromeres with those of later stages. He showed that in sharks they arise very early, and may be traced without a break through all the stages of the open neural groove into the structures that have, in later periods, been designated neuromeres. The segmental divisions extend to the anterior tip of the fore-brain, and are distinguishable in that region for a brief time after the closure of the neural groove.

He also recorded the presence of neuromeres in Amblystoma embryos with wide open neural groove, but differs from Kupffer in locating them in the neural ridges instead of the median neural plate. (See further on this point, p. 530.)

From the foregoing historical survey it appears that our knowledge regarding metamerism in the neural tube has passed through the phase of simple observation of its occurrence (Von Baer, '28, Remak, '50, Dursy, '69, and others), and has grown by successive additions to the recent conception of its segmental importance. In reaching this point, first came the work of Béraneck ('84) and Orr ('87), showing that the neuromeres of the hind-brain are definitely connected with nerves; following this it was demonstrated by Kupffer, partly, in 1886, and by McClure, definitely, in 1889, that the neuromeres extend throughout the neural tube, and that those of the trunk region merge gradually into those of the head region. Lastly, the neuromeres of the mid-brain have been especially studied by Waters ('92), the condition of that brain region having been left undetermined by previous observers. Remak was the first (1850) to suggest the segmental relations of nerves and neural segments; Béraneck the first (1884) to demonstrate it. From this time onward the definite relation of nerves and neuromeres began to be studied, and both Orr ('87) and Hoffmann ('88) are pioneers in this line of study.

It will be observed that previous to the appearance of my paper just referred to, no one but Kupffer and Froriep had claimed a very early appearance for neural segments, and these two authors had recorded their appearance only in the median part of the neural plate, and not in the neural ridges. They had not shown the structures of the open neural groove stage to be in any way connected with the neuromeres of later periods, which are present in the lateral walls of the neural tube.

In the case of Froriep's observations I think there is reason to doubt whether the segments observed are really the "neural segments" of other writers. The small number (4 or 5) which he observed in the head region does not correspond with the number of neuromeres observed by any other author in the

same region. I think, also, there is a way to bring Froriep's observations into reconciliation with my own (see p. 529).

At all events, it has been understood from the work of previous observers that the neural segments arise after the neural groove is closed, or while it is in process of closing. Waters ('92) carries the idea throughout his paper that the metameric segmentation arises relatively late, especially in the Teleosts, where he was unable to find any traces of this segmentation earlier than the ninth day of development, after the auditory pit is formed. Orr and McClure do not in every case state ages, but from their figures and the text I understand that they have not detected this segmentation in very young stages. Miss Platt mentions the fact that these segments sometimes occur in the chick while the groove is open. Rabl mentions them as being especially clear from the fiftieth to the ninetieth hour of incubation in the chicken.

I have been fortunate enough to find these neural segments in a number of animal forms in extremely young stages, and in Squalus acanthias, to trace them coherently onward into the later stages. In this form, the division of the embryo into segments takes place before the neural groove is formed, and, before any protovertebræ have made their appearance, the metameres not only extend the whole length of the embryo, but they are continued for some distance into the embryonic rim. They occur under such conditions in this animal that they cannot be interpreted as depending on mesoblastic segmentation. In Amblystoma and the newt (Diemyctylus) the metameric segmentation is present in the rudiments of the neural folds, just after their first appearance and during their period of broadest expansion. In living chick embryos of about the twentieth hour of incubation they can be made out with clearness along the walls of the beginning neural folds. It is only in Squalus acanthias, however, that I have traced the complete history of these neuromeric segments.

III. Descriptions of Stages of Acanthias.

The earliest stage in which I have detected this metameric segmentation is represented in Pl. XXVII, Fig. 25. This is an age somewhere between Balfour's stages *B* and *C*. It is, in reality, the youngest embryo of Squalus to which I have had access since I began to observe especially the metameric segments in that animal. Whether or not they occur in still younger embryos I do not know, but they are already clearly defined in the stage referred to, and it is reasonable to suppose that they may be seen in still earlier stages.

The axial embryo (Fig. 25) is just fairly established, and has reached a length of $1\frac{1}{10}$ mm. The head-end is already wider than the rest of the embryo. It has begun to show that tendency to broaden that is characteristic of the head-end of the embryo. The gastrular cavity is broad, and extends to the extreme anterior end of the embryo. In the figure it is seen even protruding beyond the head-plate. The primitive furrow, that has often been confused with the neural groove in these Elasmobranchs, is broadened at its anterior end. Fig. 63, Pl. XXIX, is a sketch of a horizontal section of this embryo to show the general appearance of the metameres in section.

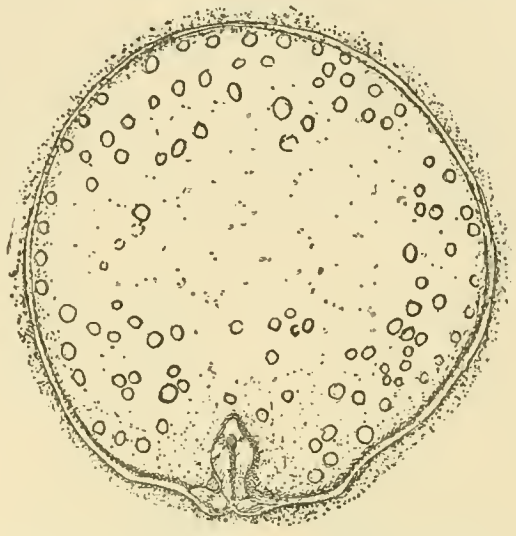
The segmental divisions in this embryo extend from the anterior end backwards along the margins of the axial part of the embryo, and out into the non-axial part or embryonic rim. There are seven or eight pairs of these segments in the embryo, and as many more, directly continuous with them, in the embryonic rim. The latter is segmented to the points where it is broken from the rest of the blastoderm. Whether or not these segments extend further into the blastodermic rim, I am unable to say.

The segments are most clearly defined along the inner margin of the embryonic rim, and extend more faintly across it. In the axial part of the embryo they are not in such a favorable position to be observed from above — they are on the rounded margins; but if the embryo be rolled into such a position that the margin is brought into view, its division into

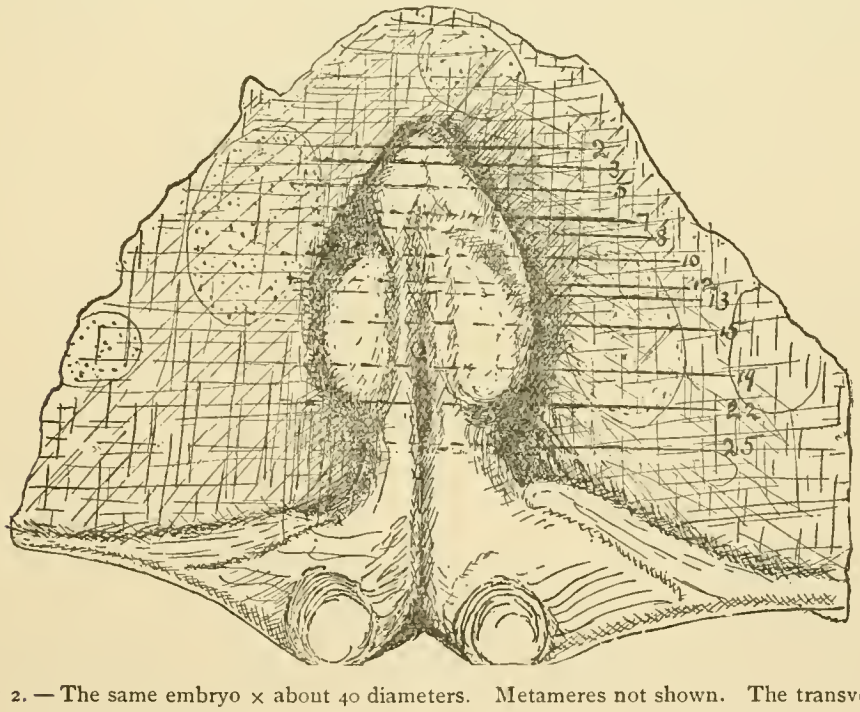
segments is more plainly seen. Near the middle part of the embryo the lines of segmentation are faintly traceable from the margins towards the median furrow. The two lines of segments are joined in front by a single median piece or segment. This unsegmented anterior tip becomes more prominent in the immediately following stages. There is no evidence to show whether this represents the primitive anterior segment or several aggregated anterior segments. These segments, once established in this very early stage, may be traced onward in an unbroken continuity until they become the neuromeres of other observers, and sustain definite relations to the spinal and cranial nerves. Ryder, in 1881, observed segmental divisions extending into the embryonic rim of Elacate, one of the Teleosts. In 1885, he figures such structures in a stage in which the neural groove is closed and the eye vesicles are well established. Although the figure shows a considerably later stage than we are now dealing with, and he does not speak of their earliest origin, nevertheless, the feature of their extending beyond the embryonic axis into the blastodermic rim agrees with my observations on Acanthias, and I think it not improbable, that Ryder's segments correspond with those I have described. These segments, observed by Ryder under such unusual conditions, have generally been interpreted by morphologists as due to precocious segmentation in the non-axial mesoderm. The segmentation I have just described is not capable of such interpretation, for sections show that the mesoderm is not yet divided into protovertebræ at this stage, and that the epiblast is the seat of the segmental divisions. The mesodermic somites of Squalus are formed later in the usual way, and the first ones appear in the trunk or neck region at a later period.

In Fig. 26 the embryo is relatively more slender in the trunk region, and there is coming to be an observable distinction between the broadly expanded cephalic plate and the narrower body. Upon the anterior end there is being formed a protruding unsegmented median tip, which is much better seen in Figs. 27 and 5. The median furrow ends in front in a broadly expanded depression. The gastrular cavity has become nar-

rowed in front by folding in of the sides, so that the embryo, when viewed directly from in front, seems mounted on a keel; the keel, however, is not solid, but contains the anterior part of the gastrular cavity, which still reaches to the anterior limit of the head. For the purpose of fixing the stages as definitely as possible, I give anatomical characteristics not immediately connected with the metameric segmentation. The embryo from which the figure was made measured $1\frac{9}{10}$ mm. in length, but there is so much individual variation in the size of embryos of apparently the same age, that the length is not very significant. There are, in that part of the embryo



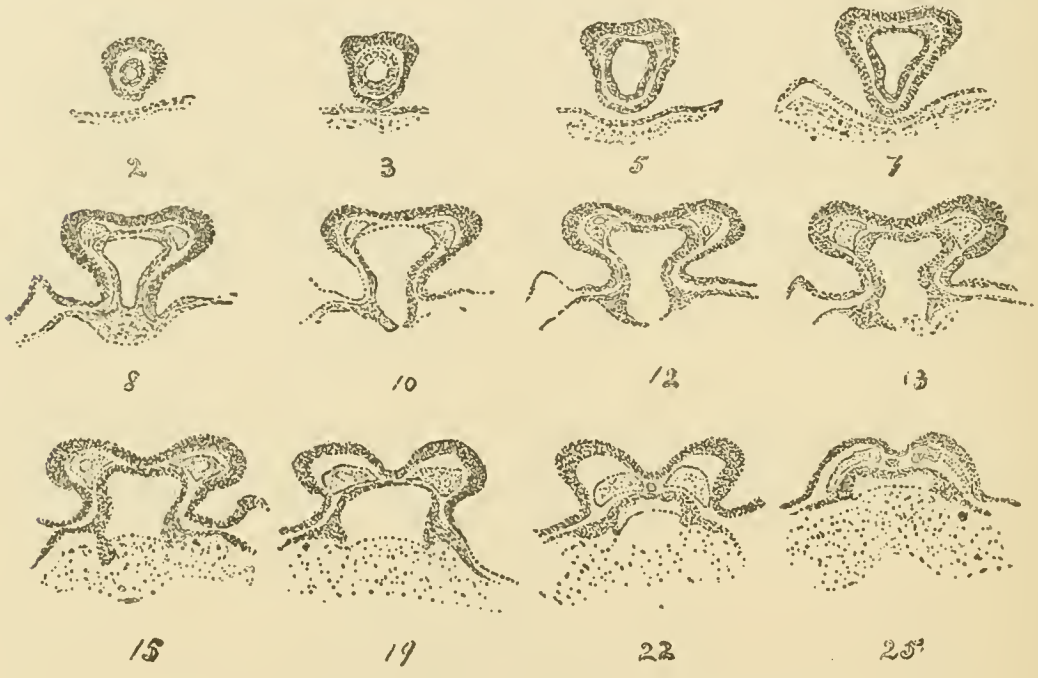
Cut 1. — Embryo and blastoderm of Acanthias just before the formation of the neural folds. × about 8 diameters.



Cut 2. — The same embryo × about 40 diameters. Metameres not shown. The transverse lines and numbers indicate the plane of the sections shown in the succeeding cut.

behind the cephalic region, three or four mesodermic somites rather imperfectly differentiated. The metameric segmentation

is very clearly exhibited along the lateral margins of the neural plate, extending from the unsegmented tip backwards, and, as in Fig. 25, is continued in the embryonic rim to the points on either side of the latter, where it is broken from the rest of the blastoderm. While it is the lateral margins that are most clearly divided into segments, in the trunk region the lines of division may be traced inwards towards the median furrow. This is probably due to the appearance of the mesodermic

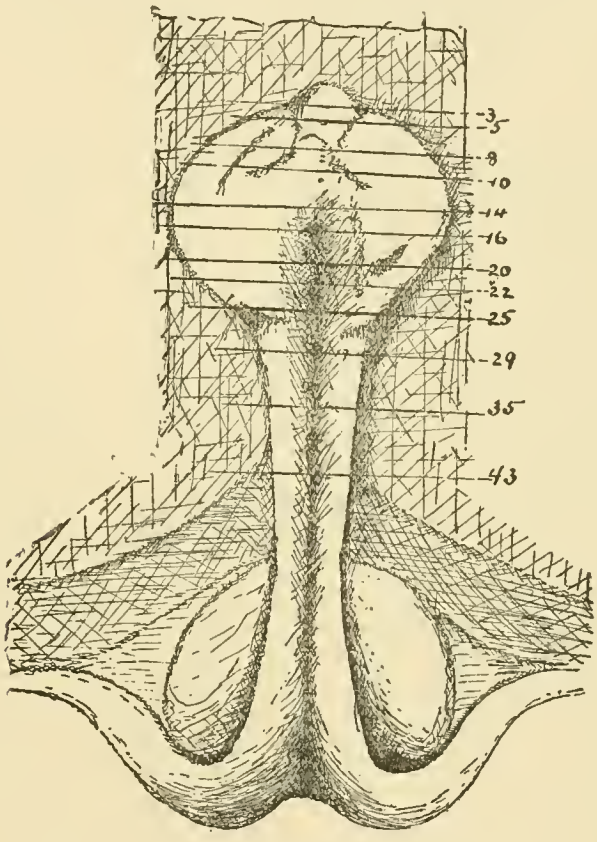


CUT 3. — Twelve transverse sections of the embryo figured in the preceding cut. × about 30 diameters. The numbers refer to the positions of the sections in the series.

somites in that region. Fig. 64 represents a horizontal section of this embryo showing metameres in the ectoblast.

Fig. 27 is in many respects similar to Fig. 26 ; it is slightly older and has reached a length of 2 mm. and shows about five mesodermic somites. Diverging furrows have appeared upon the cephalic plate that include between them a wedge-shaped central piece which terminates in the anterior unsegmented tip before mentioned. The cephalic plate is thus separated into a median and two lateral parts. It will also be noted in this figure that the lateral margins are marked off from the rest of the medullary plate by two furrows running lengthwise of the embryo, so that the plate is bordered, as it were, by marginal bands. The furrows are most distinct in the head region, but

they extend also, with less distinctness, into the trunk and fade away without reaching the hinder extremity. The furrows do not show so distinctly in cross-section as one would at first suppose, and they are in part an optical effect, arising from the way in which the neural folds are formed. This will be understood on reference to cut 5. There is, nevertheless, a distinct notch to be seen in the cross-sections of many specimens, while in others it is lacking. I am inclined, with my present light,

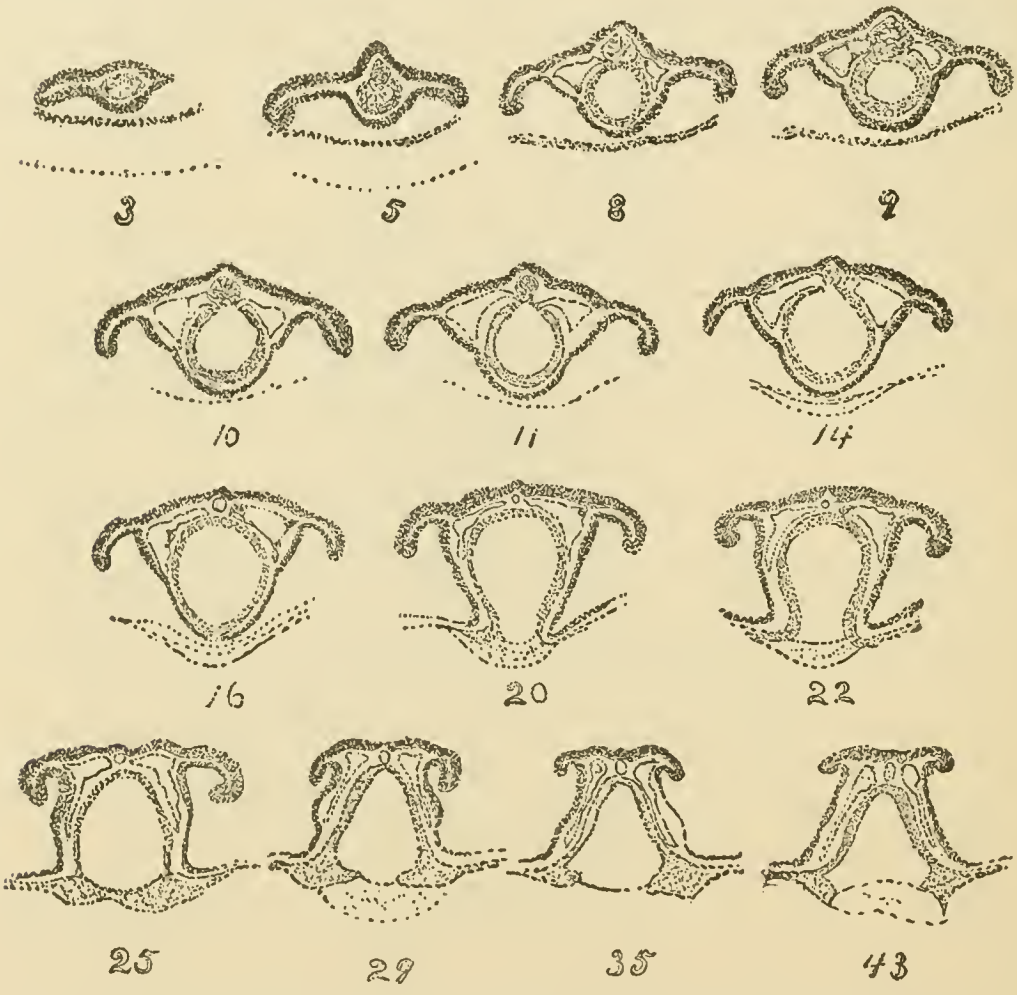


Cut 4. — Embryo of Acanthias just after the formation of the neural folds. × about 40 diameters. Metameres not shown. The transverse lines indicate the plane of the sections in cut 5.

to consider these furrows as purely mechanical effects. Sections show (cut 3) that the marginal bands, at a stage just younger than this one, are composed of an accumulation of cells forming thickened cords running along the margins of the embryo. These bundles of cells and their immediate derivatives are the material out of which the neural ridges and a large part of the medullary folds are straightway produced.

As noted in the preceding embryo, the metameres are most clearly seen from below, but the reason for this is not far to

seek. The condition of the neural folds in this animal is very unusual: when first formed they are lateral, wing-like expansions, extending along each side of the embryo, overhanging the yolk (cut 5). No sooner are they formed than they become ventrally curved, and, in this way the most clearly segmented



Cut 5. — Twelve transverse sections of the embryo of the preceding cut. × about 30 diameters. The neural folds are expanded laterally beyond the body and ventrally curved.

parts of the embryo are brought ventralwards, and this accounts for the metameres being most distinct when viewed from the ventral surface.

Figs. 28 and 29 represent two views of the same embryo; it is an older stage than that represented in Fig. 27. The neural folds are now fully formed and their ventral curvature is very marked. In Fig. 28, the optic vesicles (*op*) are seen on each side of the central tongue-like process to which attention has already been called.

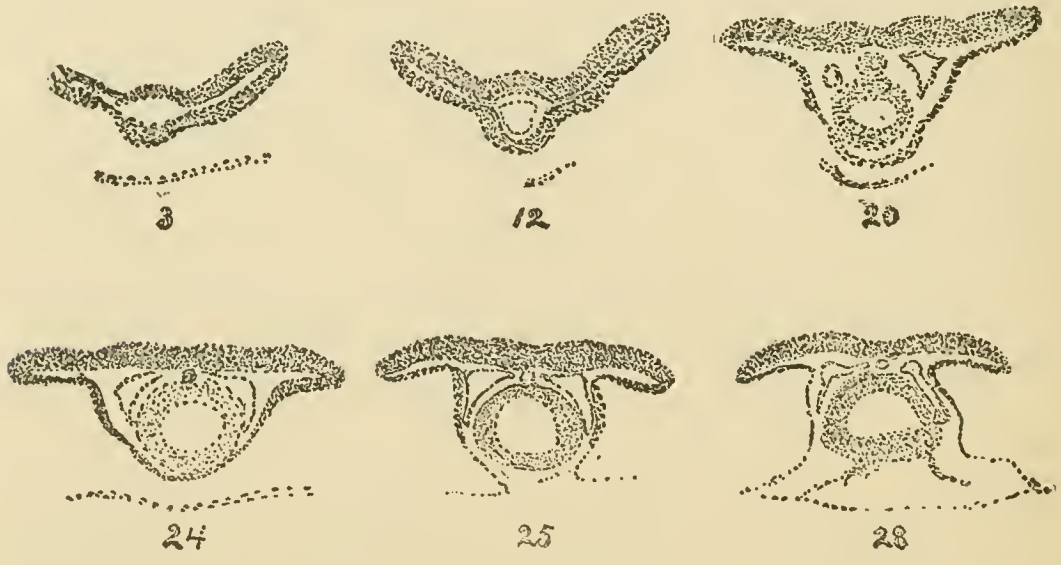
In Fig. 29,[1] the view is taken from below ; the embryo has been removed from the blastoderm and placed upon its dorsal surface and the recurved edges of the neural folds are thus brought prominently into view. This is of course the most favorable position for making observations. The dish containing the embryo should be placed over a black, non-reflecting background, and the embryo rotated into the most favorable position with a fine artist's brush.

In the actual specimen from which the figures were made the segments showed most beautifully. They appear like a row of beads running along the ventrally recurved margin, and extend with great distinctness the entire length of the embryo. Those in the trunk region are continuous with those in the head and pass into the latter without any transition forms. There is, however, some individual variation in size of the neuromeres, and they are not absolutely symmetrical on the right and left sides, but the significant thing is, there is uniformly the same number on each side in a given region, such as the hind-brain, or the brain region as a whole. Fig. 29 shows the central unsegmented piece from below with three segments on either side of it, occupying a part of the head-folds that is directed forwards. Following the beaded edge, from the head into the trunk region we find it disappearing from view beneath the expanded walls of the gastrular cavity. Viewing the same embryo from above (Fig. 28), the metameric segmentation is seen to extend the entire length of the embryo and, as in the earlier stages, laterally into its expanded parts. The segments are so plain that they may be easily counted. There seems now to be a natural landmark separating the cephalic plate from the rest of the embryo; this is an abrupt downward bending (*f*) in the medullary folds which, as I have determined, lies just in front of the future origin of the vagus nerve. There are eleven metameres in the lateral margins of the cephalic plate, including the ones embraced in this fold.

Fig. 30 represents an older stage, in which the medullary

[1] In the process of transferring and shading, the outlines in this figure have been rendered too symmetrical, giving to it a semi-diagrammatic character.

folds have unrolled from their ventrally curved position and are in the process of growing upwards. Those of the head are at this stage nearly in the horizontal plane (cut 6). The outer margins of the folds are plainly divided into segments, and the segmentation extends backwards, also, into the trunk region, but not so clearly defined. The optic vesicles are clearly seen on the head-plate, but the *accessory* optic vesicles (see p. 57) have not yet made their appearance. Beginning at the front end and counting backwards eleven segments on either side, we come to the point where the broadly expanded cephalic plate



CUT 6. — Six transverse sections of an embryo older than the preceding one. × about 30 diameters. The numbers below the sections refer to their position in the series. The neural folds are growing upwards and in section 24 have reached the horizontal plane. The depressions in Sections 3 and 12 are the optic vesicles. The embryo from which the sections are made is shown on Pl. XXVI, Fig. 7.

passes into the narrower neck and trunk. This, as before indicated, is the point of future origin of the vagus nerve. It seems to me to be a natural line of division which may be of service in determining the limits of the embryonic head. The question will be returned to on p. 543.

It should be borne in mind that all the stages so far described are very young ; the earliest ones are before the formation of the embryonic medullary folds, and the oldest one is just when the medullary folds are arching upwards to form, for the first time, a medullary groove. The mesenchymic somites have, in the interim, appeared in the trunk region, and have

produced faint surface indications in the median parts of the medullary plate.

Taking a considerable step forwards in the history of these segments, we come to the condition represented in Fig. 31. This figure shows a stage in which the medullary folds have attained a nearly vertical position; they are about to bend towards each other and meet in the median plane, but, as yet, they have not become approximated in any part of their course, and, therefore, we have an open neural groove (see cut 9, p. 553) extending the whole length of the embryo. In this figure the embryo is viewed obliquely from the right side. The rudiments of several organs have now appeared upon the head; the most anterior of these organs is the primary optic vesicle; just back of this, near the margin of the head-folds, is seen a similar elevation that represents the combined vesicle of the mid-brain and the first *accessory* optic vesicle (see p. 556). Still further back in the same line, is another similar but smaller elevation, which, I think, represents the second accessory optic vesicle. Behind the latter structure the margin of the medullary fold is bent abruptly downwards; this is a normal condition in this stage, and is of course found in the earlier stages. Back of the primary optic vesicle and somewhat between it and the mid-brain vesicle, is a rounded eminence which is the external indication of the mandibular cavity, and behind this is the branchial pouch from which the branchiæ are subsequently formed. The front end of the gastrular cavity is being cut off along with the increase in the head flexure.

Directing our attention to the margin of the right medullary fold, we note that it is clearly segmented through the head region, and backwards into the trunk region, where, in the figure, it disappears behind the yolk. The metameres extend in reality to the posterior part of the embryo. There has been a slight change in position of the foremost segments with reference to the rest of the head-plate. The three anterior ones are no longer, as in Fig. 29, on a part of the margin that looks forwards, but they have been shifted backwards, and that part of the margin that was anterior, now constitutes a part of the lateral border. Of course this shifting of position is brought

about by changes in the medullary folds. The first three metameres are, at this stage, in front of the eye, the fourth, fifth, and part of the sixth, in front of the accessory optic and mid-brain vesicle. The following five segments (seven to eleven) occupy the reflected part of the neural fold. The eleventh, as has been indicated, lies in front of the vagus nerve.

The next stage to be considered (Pl. XXVII, Fig. 32) is just after the closure of the neural groove, which is, however, still open in front by a small neuropore. The original segments are still visible throughout the head region; those in the fore and mid-brains still show from the outside. The surface-markings on the head are similar to those in Fig. 31. In front is the conspicuous optic vesicle, and behind it is another similar rounded eminence, the mid-brain vesicle. Further back there is an area, circular in outline, resembling the mid-brain, except it is not so prominent. It is, however, merely a pad of mesoderm applied to the walls of the cerebellum. There is another surface indication of considerable interest, *viz.*, a line of mesoblast running over the branchial region; it connects in front with the mandibular cavity and behind with the body protovertebræ.

Fig. 33 shows a slightly older stage than Fig. 32. The neural groove is completely closed. The extreme anterior end of the gastrular cavity has been obliterated. The cranial flexure is quite marked. This is the last stage in which the metameres are visible throughout the length of the embryo; those in the fore- and mid-brain have become indistinguishable in surface views; they are, however, still to be detected in longitudinal sections. We possess now a particular advantage in dealing with these segments, because anatomical landmarks of the head regions have become established, and these enable us to say with definiteness what are the relations of the segments to the rest of the head. This is just prior to the appearance of the auditory vesicle; when first established its center occupies the space of the segment marked 10. Sometimes, in its earliest stages, the circular area spreads over the space of the three segments marked 9, 10, and 11, but I

should say from my observations that, more frequently, it is not so widely expanded. It always settles down in Squalus acanthias, to occupy the position first indicated, and, subsequently, it is shifted backwards. The topography of the head region is similar to what it was in Fig. 32. The chief differences to note are the further differentiation of the branchial arches and clefts ; we may now distinguish the position of the future first visceral cleft, and, faintly outlined, the boundaries of branchial arches.

The segment marked 8 serves as an important landmark in all subsequent changes that affect the segments. It is seated above a depressed region in which the first visceral cleft subsequently appears, and, during all the time the segments are distinguishable from the outside, it has no nerve root.

Only a few words of description will be needed to enable us to follow the history of the metameres through the later stages. In Fig. 34, the auditory vesicle (*au*) has been formed and the first visceral cleft has broken through. The anterior metameres lying in front of the one marked 6 are no longer distinguishable from surface observation. The lines of neuromeres have been brought into contact in the median plane by the closing of the neural groove, but they are soon forced apart by the growth of the dorsal wall of the neural tube. We have now reached the stage, approximately, in which these neuromeres have been described by previous writers, — that is, the stage just after the appearance of the auditory vesicle, but it is to be remembered that Kupffer and Froriep have noted a form of segmentation in very early stages of Amphibia affecting the neural plate, but not the neural ridges (see p. 529).

In Fig. 35 some characteristic changes are to be noted ; the auditory vesicle has shifted backwards till it occupies a position opposite the eleventh metamere ; the metameres are being forced apart laterally by the growth of the dorsal wall of the hind-brain. The eighth metamere still serves as a landmark ; there are now two clearly marked metameres in front of it and three behind it. The only metameres discernible from surface view are those belonging to the hind-brain. The mid-brain is considerably increased in expanse, and the first accessory

vesicle has been crowded forwards into the region of the thalamencephalon. This may be seen after the removal of the overlying layers of mesoderm, etc. (Pl. XXVIII, Fig. 44). The fifth nerve is already well begun, and nerve fibres are also given off from the segments numbered 9 and 10.

Fig. 36 shows an embryo slightly older than that in Fig. 35. The line of neuromeres have been forced further apart by the lateral growth of the dorsal wall of the hind-brain. The ear-vesicle is no longer circular in outline, but is fast becoming a closed pouch. The eye shows the beginning of the lens and the choroid fissure. The Anlagen of the fifth, seventh, eighth, ninth, and tenth nerves are distinctly visible from surface observation. The branchial arches are all clearly outlined and the first two gill-clefts have broken through. The specimen shows about forty-five mesodermic somites. A line of surface elevations over the hinder branchiæ mark the beginning of the lateral line.

In Fig. 37 the neural segments are undergoing some changes in outline that are likely to lead to confusion in identifying them in later stages. If, for example, we look along the lower margin of the segmented border, we shall see that the elevations and constrictions are substantially as they have been in all the previous stages, but those along the upper margin no longer correspond with them. In all the preceding figures the boundaries of the segments correspond on both upper and lower margins. In Fig. 37, however, the upper margin shows elevations just above the constrictions on the lower margin. These new-formed elevations become very quickly prominent, while the segments along the lower margin lose their individuality, and the segmented area becomes more and more an irregular sinuous band with crests upon its upper margin. The entire line of segments finally becomes indistinguishable, but if they be studied in stages immediately following that represented in Fig. 37 it will be the crests along the upper margin that first catch the eye. If the observations are made from above, these crests are seen to be transverse folds on each side of the medulla, and when counted will, of course, be one less than the original segments. It is only by viewing

them from the side, and comparing them with earlier stages, that we shall be able to identify the boundaries of the original segments.

Just what is taking place during the appearance of the crests is not now clear to me. I have heretofore assumed that it signified a union of the original segments, the anterior half of one with the posterior half of the segment lying just in front of it, but at present I am inclined to question that interpretation. The crests on the upper margin are between two neuromeres as designated by Orr, and they correspond in position to his inner ridge. The point of origin (motor fibres) of the fifth, seventh, and eighth nerves (so far as it may be determined by surface view) is now clear; they arise, as Miss Platt has described them in the chicken, from the concavity (on the lower margin) between two neuromeres. This will receive fuller consideration under the heading, The Nerves. It will be interesting to note incidentally, in this figure, the very large development of mid-brain over that in Figs. 33 and 34, and the consequent crowding forwards of the first accessory optic vesicle. The latter structure is also much reduced in size, and with its fellow is in the region of the thalamencephalon.

None of these figures have shown the condition of the entire neural tube. The segmentation so clearly seen in Figs. 34, 35, and 36, represents only a part of the actual segmentation, *viz.*, that in the uppermost part of the neural tube. The rest of the tube is too much covered by mesoblast to be seen without dissection. The upper part of the tube has — in the region of the hind-brain — two thickened lateral bands of cells, which form a border on either side of the neural tube; these are the segmented parts that are visible from surface observation. I have found it necessary to remove the overlying layer of mesoblast and the outer epidermic stratum, and completely expose the walls of the brain. When thus laid bare, the walls of the neural tube show in a most satisfactory manner. The ten or twelve figures following those just described show dissections of this kind.

Pl. XXVIII, Fig. 41, shows the surface view of an embryo slightly older than that represented in Fig. 33, and Fig. 42

shows the same embryo with the overlying tissues removed from the brain walls. It is clear from a comparison of these two figures that the line of metameres seen from the surface view are those occupying the upper part of the neural tube, and that below them the entire neural tube is divided into corresponding segments. The segments do not reach across the median plane, but they are alike in number and position on both sides of the tube.

Fig. 43 represents an embryo in which the auditory vesicle is just forming. The embryo was intermediate in age between those shown in Figs. 41 and 34.

Fig. 44 shows a dissection of an embryo of the same age as the one represented in surface view in Fig. 34.

Fig. 45 is taken from an embryo just younger than that represented in Fig. 35. By the growth of the dorsal wall of the hind-brain the line of metameres have been forced apart. This expanded dorsal wall, being a new growth, is not divided into segments.

Fig. 46 represents a slightly older embryo with the optic vesicle and also the auditory vesicle removed. There are in this figure eight segments in the hind-brain that show plainly, and faint indications of a ninth.

Fig. 47 represents a dissection of the embryo from which Fig. 35 was made, and therefore a direct comparison can be made between the two figures.

Fig. 48 shows the condition of the brain walls in an embryo just older than that represented in Fig. 37. From the continued growth of the dorsal wall the metameres on each side have become widely separated. The ear-capsule has not been removed.

Fig. 49 represents a slightly older embryo than the foregoing one. The auditory and the optic vesicle have been removed. There are now distinctly nine segments in the hind-brain region. The U-shaped segment, No. 12, in the hind-brain lies opposite the ear-capsule. This figure shows well the condition of the neuromeres described in Fig. 37, in which there is no longer (as in earlier stages) a correspondence between the ridges on the upper margin and those on the lower margin of the segmented lateral bands of the neural tube.

Up to this point the figures described have all been magnified uniformly 45 diameters; but on account of the increase in size of the embryos it will be better to carry forward the history of these segments with figures drawn on a smaller scale. Accordingly Figs. 50–60, inclusive, are magnified only ten diameters.

Fig. 50 represents an embryo of the same age as that shown in Fig. 32.

Fig. 51 shows the entire embryo, partly dissected, of which Fig. 43 is a portion more highly magnified. Behind the figure is seen the line of fusion of the lips of the blastopore.

Fig. 52 is a sketch of the embryo of which Fig. 47 is the enlarged view of a partial dissection. They all show well the segmented condition of the walls of the hind-brain.

Soon after the age represented in Fig. 55 is reached, the neural segments fade away. Figs. 57 and 60 (Pl. XXIX), represent the head region of older embryos in which the segments are no longer visible.

Taken together, the figures now described give a comparatively full view of the neural segments in different ages. They show them from their first appearance to the time they fade away. We learn from this examination that the neural segments are established before any embryonic organs appear, and that in the early stages they extend not only throughout the length of the embryo, but into the embryonic rim. In the earliest stages the segments are alike, and there is no structural distinction to be made between those in the head and those in the trunk, or even those in the embryonic rim.

In sagittal sections the neuromeres are well exhibited. Fig. 72 shows a section of a specimen just after the closure of the neural groove in which the five neuromeres belonging to the fore- and mid-brain are exhibited. The second neuromere coincides in the median plane with the neuropore. This is also to be seen in surface view in Fig. 32. Very soon the anterior brain regions become so much modified and expanded that the original segmental divisions are no longer visible.

Figs. 66–71 (Pl. XXIX), show six successive sections of a specimen slightly younger than the one just described. In

front are seen three of the brain vesicles, — those of the fore-brain, the mid-brain, and the cerebellum. The thalamencephalon and the prosencephalon do not show as separate parts. In the region of the hind-brain are seen three neuromeres especially well developed. They present the appearance of three bars; they are the seventh, eighth, and ninth neuromeres respectively. The other neuromeres are present, but they do not stand out with such distinctness as the three mentioned. When the ear vesicle first arises it makes its appearance opposite the ninth neuromere.

Figs. 73, 74, and 75 are sections of a somewhat older embryo after the ear vesicle is established. In these figures eight neuromeres of the hind-brain are visible. The ear vesicle is opposite the tenth neuromere. Just in front of it, in Figs. 73 and 74, are the roots of the eighth and seventh nerves, respectively, those from the former nerve being connected with the tenth neuromere, and those of the latter with the ninth neuromere.

In Fig. 75, the fifth nerve is seen to have connections with the first and second neuromeres of the hind-brain, *i.e.*, the sixth and seventh neuromeres respectively.

As already indicated, the eighth neuromere bears no nerve, and Hoffmann remarks, "This seems to be the case in all Vertebrates."

IV. Supplementary Observations on Other Animals.

I have also made some supplementary observations on these neural segments in Amblystoma, Diemyctylus, Rana palustris, Torpedo ocellata, and the chick. In all of these forms the metameric divisions are to be found in very early stages before any of the embryonic organs have been formed; and they are in all essential features like those I have described for Squalus acanthias.

Figs. 113 and 114 (Pl. XXX) show camera sketches of an Amblystoma egg, with broadly expanded neural plate and widely open neural groove. The neural folds or ridges are divided throughout their length into a series of segments with no espe-

cial distinguishing features between those of the head and those of the body region. The median plate included between the neural ridges is smooth at this stage; at a slightly later period, however, while the groove is still widely open, the median plate exhibits very faint transverse markings.

The contrast between this condition in Amblystoma and that in a closely related egg (Rana palustris) is very instructive. In the latter (Fig. 115) the cephalic plate is very obviously segmented, while the folds in the neural ridges are extremely difficult to see. We thus have in these two closely related eggs strikingly different conditions. In the one it is the median plate material that is thrown into obvious folds, while those in the neural ridges are well-nigh indiscernible; and in the other the conditions are reversed. This is not, however, to be taken as indicating profound differences; for a little careful observation shows that the median divisions do not correspond to those in the neural ridges, and therefore the median folds in Rana palustris are not to be compared to the primitive segmental folds in the neural ridges of Amblystoma. Careful observation shows, also, that there are segmental divisions in the neural ridges of Rana palustris that do correspond, in number and general characteristics, with those in the neural ridges of Amblystoma. These latter segmental divisions are extremely difficult to see in Rana palustris. There are four or five median transverse divisions in the cephalic plate of that form, while there are ten or eleven segments in the neural ridges of the same region.

These facts throw light upon the apparent discrepancy between the observations of Kupffer and Froriep and those I published in a preliminary article. Both the former authors observed segments in the median plate of amphibian embryos, and none in the neural ridges, while I have figured segments in the neural ridges of Amblystoma, and none in the median plate. But my later observations show that the appearances even in closely related eggs of the same age are not necessarily identical.

Froriep agrees with Kupffer as to the position of the segments in the early stages, but not as to their number. In

Salamandra maculosa he found only four segments in the head region, while Kupffer found eight in the same region of Salamandra atra (Fig. 117). All other observers have found eight or more in the head region, and Froriep stands alone in identifying so small a number of segments in the head. In Rana palustris there are about four obvious divisions in the median plate of the head (but, in addition, there are other segments in the neural ridges to the number of ten in the same region). This affords a suggestion that the segments observed by Froriep (see Fig. 116) correspond to those I have observed in the median plate of Rana palustris, and not to the segments in the neural ridges, which are evidently homologous with those in the same position in Amblystoma, Acanthias, and other forms.

Whether we find the median plate smooth in Amblystoma or faintly segmented depends upon the stage at which the examination is made, and we recognize that the appearances in any one egg are not constant throughout the open groove stage; and, further, that eggs of closely related animals are by no means necessarily similar at corresponding stages. The failure to take things of this nature (which are really common enough) into consideration has, I think, given origin to many differences of opinion and formed the basis of many a controversy. This should make us careful in comparing results to have the same material in precisely the same stages.

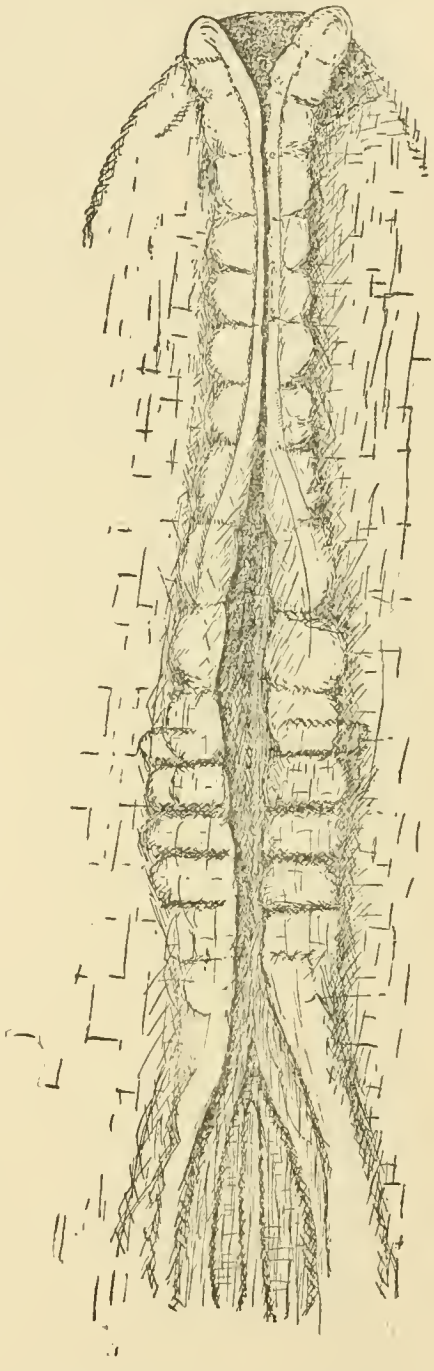
In Diemyctylus the conditions are very similar to those in Amblystoma. The neural folds show metameric divisions in the stages with wide-open neural groove. In the three amphibian forms I have examined there are about ten pairs of segments in the broadly expanded neural folds of the head.

In Torpedo ocellata I have also noted the occurrence of this metameric segmentation in several stages. The youngest embryos of that animal I have had corresponds to the stage designated "C" by the Zieglers, and beginning at that point I have traced it along through several stages with a widely open neural groove. This form is so closely related to the one I have especially studied that we would naturally expect — as is the case — marked similarity between the neural segments.

Torpedo is not so favorable for the study of the segments as Acanthias. The folds are much fainter in the former than in the latter, but the significant thing is, that the number in a given region in Torpedo corresponds to that in Acanthias.

In the chick these segments may be detected as soon as the neural folds are established, before there is any trace of protovertebræ. A small number is visible in the blastoderm of the twelfth hour of incubation just as the head-folds are first outlined. Their history has been carefully traced by one of my students, Mr. F. A. Hayner, and it agrees in all essential features with the history of the same segments in Acanthias. Cut 7 shows the appearance of these segments from surface view in a chick embryo of about twenty-four hours incubation. There are eleven segments in front of the first-formed protovertebræ. The cell-arrangement in the metameres, as shown in sections, is the same as that described by Orr in neuromeres of older stages of the lizard (Anolis).

From these supplementary observations it is clear that the occurrence of primitive neural segments, as the first definite expression of metamerism, is not an isolated case in Squalus. They occur in the same very early period in all the other forms examined, and in all of them precede any division of the mesoderm into protovertebræ.



Cut 7. — Embryo of chick with four mesodermic somites × about 45 diameters from a sketch by Fred. A. Hayner. The neural folds are divided throughout their entire length into primitive metameric segments, while there are but four mesodermic somites found.

V. General Considerations.

1. *New Aspect of the Problem of Metamerism.*

The neural segments have now been shown to occur in extremely young stages of a number of animals. The fact of their presence in these early stages once established they assume new importance. They have too definite a history to admit of being set aside as mere beadings or undulations of no metameric significance. When I first began, some two years ago, to notice these segments in extremely young embryos, I attached no particular significance to them ; but a comprehensive study has convinced me of their segmental importance and everything taken into consideration they furnish, I think, a more satisfactory basis for interpretation of metamerism of the head than we have had before. I have pointed them out to several observers, in Amblystoma and the chick, and have found no one who had ever noticed them before ; but through the kindness of others I have had my observations as to the number in the head region confirmed. Since these structures have been overlooked in the earliest stages, it will not be out of place to say a word about their general appearance, and how to see them.

It is extremely difficult to represent them on paper just as they look. All my drawings are camera tracings, and so far as number and arrangement of the segments are concerned, have been verified and reverified over and over again. They are a little too distinct in some of my drawings ; in making the figures clear enough so there shall be no doubt as to what I mean, their distinctness has been somewhat exaggerated. On Pl. XXVI I have given a few photographs from untouched negatives that show these segments more as they appear in the actual specimens. To observe them successfully is largely a question of getting shadows. One can look directly at the crenated margins without seeing the segments at all, but by tilting and rotating the specimens until the proper angle of illumination is found, they may be seen with more or less distinctness, according to the general state of preparation of the

material. I have never seen a shark embryo, of the proper age, of any method of preparation, in which I could not detect them. A dead-black background is, of course, the best surface for observing anything of this kind by reflected light. A white surface is occasionally recommended, but it is desirable to cut off all reflected rays except those coming from the specimen, and one may see delicate structures on a black surface that cannot be seen at all on a white background.

The care with which the specimens are prepared makes a great difference in the clearness with which these structures may be seen. The conditions under which most material is hardened are unfavorable. There is usually an albuminous fluid in contact with the embryo, and this, together with minute fragments of yolk, coagulates when the fixing reagent is used, and forms a coating over the embryo. The embryos should be washed by a very gentle jet of the reagent immediately after their immersion, and the clouded reagent should be removed and replaced by clear fluid. This makes the preparations beautifully clear.

2. *Are they Artifacts?*

There is one question that must be answered for all new structures, *viz.*, are they artifacts — produced by the reagents used? Too great precaution cannot be taken in considering this question.

I have used the following reagents in preparing my material: picro-sulphuric acid, picro-nitric acid, Flemming's stronger solution, Davidoff's corrosive-acetic, chromic acid with a trace of osmic, corrosive sublimate removed with iodine. I have observed Acanthias material prepared by all these various methods, and in every case have found the segments as I have described them. I have always taken the precaution to count the number in the head region, and uniformly have found the same number of segments there, regardless of the hardening reagent used. I have had a complete parallel series of specimens hardened with Davidoff's corrosive-acetic and Kleinenberg's picro-sulphuric, and have compared the two, step by step,

one set serving to fully corroborate the observations made on the other.

In Acanthias I have followed the history of these segments very carefully, and have traced the earliest-formed ones *without a break* into the later stages, and identified them with the neuromeres. If, therefore, the segmental folds of the open neural groove stage are artifacts, it may with equal force be claimed that the so-called neuromeres, which are later stages of the same segments, are also artifacts.

It should also be borne in mind that similar segments exist in correspondingly early stages in Amblystoma, Rana palustris, Diemyctylus, and the chick. It is truly interesting to observe the way in which these different kinds of material bear out the interpretation, that we are dealing with veritable anatomical structures.

The most satisfactory reply to be given to the question is based on study of fresh material. Fortunately, the chick offers at all times a source where we can get living embryonic material at any desired age. I have repeatedly observed these segments in living chick embryos[1] in the eighteen to twenty-two-hour stages, and have treated them with reagents while they were actually under observation. The effect of the addition of picro-sulphuric acid is to render immediately the walls of the neural groove opaque and more clearly defined, but not to affect the number or arrangement of the segments. Camera sketches have been made before the addition of the reagent and compared with those made after the specimens were hardened. The two correspond, as regards number and arrangement of the segments. I have also noted these structures in living embryos between the twelfth and fifteenth hours of incubation. Their consecutive history has been traced in my laboratory by one of my students, and the results may be expected to appear later in this JOURNAL.

I have likewise observed them in living embryos of Amblys-

[1] The embryo was removed with a part of the blastoderm to normal salt solution, which was kept at the proper temperature, and the specimens tilted and rotated with a very fine sable brush over a black background. The structures are, of course, faint, and delicately, although definitely, outlined.

toma in the open neural groove stage and have compared them with hardened specimens of the same age.

3. *Do they afford a Clew to the Metamerism of the Brain?*

If we grant that these are truly segmental structures, the question still remains : Do they afford the best or even a good clew to the number of segments in the primitive brain ? If so, they must be shown to be equally important in this direction with myotomes, branchiæ and cranial nerves.

In estimating the claims of these various forms of segmental divisions, to rank as primitive, the question of the time of their appearance in developmental history will be significant. On this point I wish to observe, that in all forms I have studied — embracing representatives of Birds, Amphibia, and Selachians — the neural segments are among the first anatomical structures to be established ; before the vestiges of any organs have appeared, the embryo is divided throughout its length into similar segments. These metameric divisions, therefore, antedate myotomes, branchiæ, cranial nerves, or any other structures that exhibit metamerism. They persist through the early stages of development, and become definitely related to segmental nerves and segmental sense-organs. In the light of their very early appearance and their history, I think we are justified in saying that they are the most satisfactory traces of primitive metamerism that is presented in the group of Vertebrates. They may be looked upon as a survival of that general segmentation, which all agree in assuming for the (not too remote) ancestral form.

It should also be observed that the entire embryo is segmented, and the term "Metamerism of the Head" should be understood to signify merely regional metamerism and not a different kind of segmental division from that occurring in the rest of the embryo.

The study of sections shows that the neural segments are distinctly differentiated groups of cells, and not merely a series of undulations. As has been already pointed out the arrangement of cells is like that described by Orr for the neuromeres in Anolis.

4. *They are formed Independently of Mesodermic Influence.*

The next point I wish to maintain with regard to these segments is that they are formed independently of mesodermic influence.

In a preliminary account, I went so far as to state that the segmentation is epiblastic, but that statement should be qualified. It is certainly most clearly expressed in the epiblast, but the other germ-layers apparently feel the same influence, and partake of this segmental division in a modified degree. It is least clear in the mesoderm — in sections it may be made out in this layer, and also in the endoderm. The so-called neural segments are developed throughout the entire length of the embryo before any protovertebræ are formed and, therefore, they must be independent of any formative influence of the latter.

There is also a notable difference in the two forms of metameric division ; the formation of protovertebræ, as is well known, proceeds from a certain point forwards and backwards, and if the segmentation I have described were moulded over the former, we should expect the neural segments to appear gradually, keeping pace with the formation of protovertebræ.

Nevertheless, the majority of authors who have touched upon the question have taken the position that segmental division of the mesoderm is primary and that the neural segments are moulded over it, but all this time the early history of the neural segments has not been known. Kupffer announced, in 1885, that segments appear upon the median neural plate of Salamandra atra while the groove is widely open and before the appearance of any protovertebræ. He designated this primary metamerism. Seven years later, Froriep ('92) studied the question of metameric segmentation upon closely related forms (Salamandra maculosa and Triton cristatus), but reached different conclusions as regards the nature of the segments.

Froriep's conclusions are based on both surface study and sections. He shows in very young Triton embryos a segmented condition in the neural plate included between the

incipient neural folds. He expressly states that the segmental divisions do not extend beyond the plate into the neural ridges. The latter, he says, become segmented later ; only, when in the process of closing of the neural groove, they are brought directly over the originally segmented plate, and are made to feel the effect of the underlying segmented mesoblast. The number of segments which he detects in the neural plate at this period is very small. He finds only four in the greatly expanded anterior part.

My observations (already given on p. 530), differ from his in a fundamental way: showing, in Amblystoma, Rana palustris and the newt, the presence of unmistakable segments, in the earliest formed neural ridges, and not less than ten pairs in the broadly expanded head-plate.

In Squalus acanthias, also, it is the incipient neural folds that are the most plainly segmented, and in this animal they are so situated that they cannot possibly come under the influence of the mesoblast in any mechanical way. It will be remembered from descriptions of Figs. 27 to 30 and from sections of the same (cuts 3, 5, 6) that the neural folds are formed as wing-like expansions, extending laterally beyond the body. The mesoblast does not accompany these outfoldings of the epiblast, and when they best exhibit the segmental divisions no mesoblast enters into them.

As for the rest, Froriep gives figures of sections to show that the mesoblast is at this very early period actually divided into somites that correspond to the external segments. His figures certainly show segmental divisions, but being wood-cuts they are not entirely satisfactory when the question arises whether they are really protovertebræ. Froriep interprets them as such and closes his article as follows : "Die Gliederung des Wirbeltierkörpers ist ursprünglich an das mittlere Keimblatt gebunden ; wo sich als Produkte des äusseren Keimblattes segmentale Anordnungen finden, sind dieselben durch Anpassung an die Metamerie des Mesoblastes sekundär entstanden."

The internal condition figured by Froriep is interesting when compared with that in Squalus acanthias at corresponding stages. I have found in that animal an undulated condition of

the mesoblast, but it is not clear in my mind that we are justified in looking upon the divisions as primitive mesoblastic somites ; before seeing Froriep's paper I had considered them as undulations, probably due to the same influence that has thrown the epiblast so clearly into segments. In many embryos of Squalus, showing epiblastic segmentations, they are, so far as I can determine from sections, entirely lacking. It seems to me that they are rather to be looked upon as a consequence of primitive segmentation and not as protovertebræ. This view is substantiated by the fact that the formation of the latter takes place in the usual manner at a later period.

Considering the late stages in which the neuromeres have generally been described, it was a natural inference that they arise after the neural tube is established. Minot, in expressing his conception of the formation of the neural segments, based on the descriptions of Orr and McClure, says : "Their appearance seems to depend on the development of the primitive segments of the mesothelium. When the segments are fully formed, and before their inner wall has changed into mesenchymal tissue, they press against the medullary tube and oppose its enlargement ; at least one sees that the tube becomes slightly constricted between each pair of segments and slightly enlarged opposite each inter-segmental space." But the neural segments appear so much earlier than the primitive segments of the mesothelium, that this interpretation is no longer tenable.

The facts made known will tend to materially modify the current theory of metamerism, which assumes as fundamental that metameric divisions begin in and depend on the mesoblast. As Adam Sedgwick says, in a recent paper : "The segmentation which obviously persists in the trunk region, and which begins with segmentation of the mesoderm, and is moulded upon it in the manner characteristic of all metamerically segmented animals." It seems to me a strained inference, that the middle layer — the last germ-layer to be formed — should be the bearer of this primitive metameric division, but, of course, the final appeal must be made to sections and my sectioned material has given evidence entirely corroborative of

the surface observations; sections of the embryo illustrated in Fig. 25 show that the mesoblast is nowhere divided into segments, while the epiblastic somites extend throughout its entire length. When the stage represented in Fig. 26 is reached, the first mesoblastic somites (three in number) are to be detected, in the trunk region; the rest of the mesoblast remains undivided, but the epiblastic segmentation is as before. I have also made a careful study of the sections of the chicken embryo illustrated in cut 7, and can find no segmental divisions of the mesoblast outside the protovertebræ (five in number), but the metameric divisions of the epiblast extend from the extreme anterior tip back to the point where the neural folds fade out.

5. *Presence of these Segments in Embryonic Rim and Primitive Groove.*

Another point to be noted regarding these primitive segments is their range. They do not end at the posterior limit of the axial embryo, but extend on either side into the embryonic rim. As the embryo increases in length, those in the embryonic rim are apparently drawn into the axial embryo; at any rate, I do not find evidence of extension by budding in the axial embryo, and, in the early stages, the number of neural segments therein increases in direct ratio to the lengthening of the embryo. This would indicate that at least some of the material of the embryonic rim contributes to the elongation of the embryo.

In the chick the walls of the primitive groove are also divided into segments that are similar to those that appear in the neural folds.

6. *Number of Segments represented in the Brain.*

There is considerable variance in the conclusion of different observers as to the number of neural segments represented in the brain. Orr, McClure, Waters, agree in identifying six in the hind-brain of the chick and lizard, and five in the hind-brain of Amblystoma. In estimating the entire number in

the brain, there is to be added, according to McClure's count, four for the combined fore- and mid-brain, and, according to Waters', five for these regions. Thus, McClure considers the brain to represent a total of ten neuromeric segments, and Waters makes a total of eleven segments.

The European investigators, in general, have found a larger number of these segments in the hind-brain, and usually have not observed them in the fore-brain. Dohrn gives eight in the bony fishes, Rabl seven or eight in the chick, Hoffmann seven in Reptilia ; Froriep counted twelve in the entire brain of mole embryos, and Zimmerman gives thirteen in front of the vagus. The fore-part of the brain has been regarded as non-metameric, and the question of the anterior extension of segmentation is an important one. In regard to that point, the evidence furnished by the neural segments, shows that the segmentation extends throughout the fore-brain. This corresponds with the investigations of Whitman on the nervous system of Clepsine, in which he shows conclusively that the cerebral ganglia of that animal belong to the metameric series.

Recent observations on a different set of segments, *viz.*, segmental divisions in the mesoderm of the head, have brought to light a relatively large number of cephalic segments. Dohrn, in 1890, observed eighteen or nineteen mesodermic segments in the head of Torpedo marmorata of 3 mm. in length. Killian ('91) in the following year describes seventeen or eighteen in Balfour stages *F* and *J* of Torpedo ocellata. He also makes a correction to Dohrn's enumeration, making it correspond with his own. In later stages of Selachians, as Van Wijhe has shown, they are reduced to nine, or to ten according to Miss Platt.

There is such a fundamental relation between muscle and nerve that we might expect myotomes and neural segments to bear a direct numerical relation. My observations show a smaller number of actual segments in the brain-walls than that arrived at by Dohrn and Killian through studying the myotomes of the head region. In the hind-brain I have found eight segments represented in the earlier stages, and a ninth segment which, to all appearances, belongs to those of the spinal cord, but later this ninth segment becomes clearly associated

with those of the hind-brain. I have taken this to signify that the hind-brain has encroached on the territory of the spinal cord, and has embraced at least one segment originally belonging to the cord. It is reasonable, on theoretical grounds, to suppose that such a process has taken place and has been several times repeated.

In order to bring the segments of the brain into satisfactory evidence I have completely laid bare the brain-walls by removing the overlying layers of mesoderm and epidermis. The segments after being exposed in this way stand out so clearly that I feel considerable confidence in my count of them in Squalus acanthias. In addition to the nine segments in the hind-brain, I have found five in the combined fore- and mid-brain. In the latter particular I agree with Waters, but, of course, differ as regards the total number in the brain. McClure says (p. 39): "I will show that Dr. Hoffmann is probably wrong in considering the hind-brain as consisting of seven segments, and that the segment considered by him as the first segment of the hind-brain is rather the posterior segment of the mid-brain; in other words, it is the second neuromere of the mid-brain." The recently adduced evidence is all in favor of Hoffmann's observation. According to my observations, there are still represented in the otogeny of Squalus at least fourteen paired neural segments belonging to the brain region. There should be added to this enumeration the median unsegmented tip which terminates the line of segments in front, and, as stated above, there are reasons to suspect (although no direct evidence in that structure) that there may be more than one segment included in that terminal piece. If the terminal piece represents a single pair consolidated, then there are fifteen neural segments in the earliest condition of the brain of Acanthias.

7. *Relation of the Neuromeres to Sense-Organs and Cranial Nerves.*

The sense-organs and cranial nerves, undoubtedly, at first sustained definite segmental relations to the neural segments. In the spinal cord there is still a pair of nerves for each neuro-

mere, but in the brain the primitive relations have been greatly modified or obliterated. There is not sufficient evidence to show what might have been the primitive arrangement in that region. Of course, we bear in mind that the sensory fibres grow centripetally, but if they appertained to a particular segment, we might make a tentative estimate of numerical relations as follows : —

I. First Neuromere of Fore-brain, Olfactory.
II. Second Neuromere of Fore-brain, Optic.
III. Third Neuromere of Fore-brain, possibly nerve to Pineal Sense-organ ?
IV. First Neuromere of Mid-brain, Oculo-motor.
V. Second Neuromere of Mid-brain, Trochlearis.

It is easier to assign relations for the segments of the hind-brain. In my assignment I agree substantially with Hoffmann.

VI. First Neuromere of Hind-brain, anterior root of Trigeminus.
VII. Main root of Trigeminus.
VIII. Third Neuromere of Hind-brain ; no nerve root, at least in early stages.
IX. Facialis.
X. Auditory. The roots of the Facialis and the Auditory arise separately in Squalus acanthias.
XI. Glosso-pharyngeal.
XII, XIII, XIV. Roots of the Vagus.

At the time of its first appearance the auditory saucer lies in front of the tenth neuromere, but is soon shifted backwards opposite the eleventh.

Minot has directed attention to the fact that Miss Platt and McClure ignored the difference between ganglionic and medullary nerve-fibres, and this is important ; but the ganglion ridges are divided in the same way as the neural tube, and we may speak of the neuromeres as including the segments of the ganglion ridge.

The relation of the nerve-fibres to the neuromeres will be considered more in detail under the heading of The Nerves.

8. *Head and Trunk.*

It would be a great convenience to anatomists to have some means of distinguishing between the head and trunk of very young embryos. It is generally regarded as impossible, on account of the lack of definite landmarks, to assign such a line of division, in early stages, before the origin of the auditory vesicle. As Sedgwick says, in the article referred to above, "The term head here must be regarded as meaning the anterior end of the body, for it is not possible in these young embryos to distinguish the head from the trunk." Nevertheless, in the young embryos of Squalus acanthias, there seems to be a natural line of division. The neural folds in this animal run forwards with the margins nearly parallel to one another, and then expand in front into the broad cephalic plate. The expansion takes place rather abruptly, and it is possible, in very young stages, to draw a line indicating where the expanded part of the cephalic plate joins the non-expanded part of the embryo. This line may be drawn without any reference to the number of primitive segments that it will cut off. The position of such a line is indicated by *AA* in Fig. 26. This is, in Squalus acanthias, just in front of the point where, subsequently, the vagus nerve begins. As before indicated, there are uniformly eleven neural segments in front of this line. Their number remains the same from the earliest stages until the anatomical landmarks (auditory vesicle and nerves) appear that enable us to determine the limits of the head region. In this animal, we may identify that part of the head that lies in front of the vagus nerve by counting the first eleven neural segments. It will be merely a question of agreeing upon the number of primitive segments belonging to the vagus, to enable us to locate with definiteness the hindermost limit of the head. Besides being of use in other ways, this would enable us to say, even in the earliest stages, what is head-mesoblast and what is trunk-mesoblast.

It is interesting, in this connection, to notice that there is in Amblystoma a similar greatly expanded cephalic part with an assignable line of its union with the non-expanded trunk

region, and there are also in this form ten or eleven segments included in the expanded head part. In the chick there are eleven segments in front of the first formed protovertebræ.

9. *Summary.*

Some of the more important facts and conclusions regarding the neural segment may be briefly stated:

1. They are natural structures, not artifacts produced by the reagents. This has been shown by their constancy in appearance and general characteristics when different reagents are used; their consecutive history; their similarity in different kinds of embryos; and lastly, by their presence in fresh material before any reagents have been used.

2. *Early appearance.* — The so-called neuromeric segmentation is the first to appear. It arises long before there are any segmental divisions of the mesoderm, and therefore cannot depend upon segmental divisions of the middle germ-layer. Neuromeric segmentation is more primitive than mesodermic segmentation.

3. *Structure.* — The cells in these segments are characteristically arranged even in the earliest stages, and their arrangement and their structure would indicate that they are definite differentiations of cell areas, not merely mechanical undulations.

4. *Extent.* — The entire embryo is divided into similar segments, and passes through an arthromeric condition similar to that of arthropods and worms. In Squalus the neural segments extend also into the germ-ring, and in the chick at times into the primitive streak.

5. *Number.* — There are in Squalus acanthias eleven segments in the brain region in front of the vagus nerve, and fourteen paired segments in the entire brain region, as follows: nine in the hind-brain, two in the mid-brain, and three in the fore-brain; this does not include the anterior tip, which may represent several consolidated segments.

6. *Backward growth.* — There is some evidence to show that the spinal cord region is being encroached upon by backward

differentiation. Early in the history of these segments there are seven that clearly belong to the hind-brain, and later there are successively added two more.

7. *Nature, position.* — These segments are clearest in the epiblast; the other layers are slightly affected by the segmental influence; the mesoblast least of all. The segments are serially homologous.

8. *Relations.* — They are directly (if not genetically) related to the cranial and spinal nerves. The neural ridges are divided in the same manner as the neural tube. The segments are also directly related to the sense-organs through nerves.

9. *Transformations or modifications.* — The modifications are most extreme in the anterior part with the early obliteration of those belonging to the fore- and mid-brains. Those in the hind-brain region are clearly defined for some time after the establishment of the cranial nerves, and then they fade away.

This is about all that can be said about their transformations, for the modifications have not yet been worked out in detail.

PART II. — THE SENSE-ORGANS.

The sense-organs of Vertebrates embrace all those structures that are endowed with special sensibility. They differ from one another mainly in degree of differentiation, and form a series, at the lower end of which are simple sensory papillæ, and at the upper end are complex organs like the eye and ear. We recognize two orders of sense-organs — simple generalized ones, the ganglionic sense-organs, and more specialized ones belonging to the so-called five senses.

The question of the origin and relationship of these sense-organs is full of interest. From the combined results of investigations on both Invertebrates and Vertebrates it seems altogether probable that the higher sense-organs have been derived from those of a lower order, and, indeed, that they have all been differentiated from a common sensory basis, and are therefore related in a direct way.

This view has been entertained by morphologists for upwards of ten years, and has a firm foothold in philosophical discussions. The evidence favoring such an interpretation has been accumulating, and although still incomplete, the hypothesis was never so well supported as at present.

Professor Whitman's researches were among the first to bring out this conception. Ten years ago he demonstrated in leeches the genetic relation between eyes and tactile papillæ, and since that time it has no longer been a matter of pure assumption to say that certain end-organs are modifications of a common sensory basis. He shows that the sensory papillæ located on each body-ring in the leeches have a very interesting relation. In the hinder part of the body the papillæ are purely tactile, but passing headward they become gradually modified, and are at first mixed visual and tactile, and finally the anterior ten pairs are purely visual. We have thus had for upwards of ten years a well-authenticated case of the derivation of sense-organs of a higher grade from those of a lower order.

In a more recent publication, "The Metamerism of Clepsine," 1892, Professor Whitman has added to the definiteness of his earlier discoveries by observations on a new species, *viz.*, Clepsine hollensis. He shows by a comprehensive study of the elements of the nervous system, the nerve distribution, and the sensillæ the complete homodynamy of all the segments.

Regarding the relationship of the eyes to other metameric sense-organs, he says: "One more peculiarity of this species may be noticed. The median rows of sensillæ are quite well developed as eyes, at least on segments IV, V, and VI. The visual elements in segment IV are quite numerous, and they are placed in a pigment cup quite like that of the principal eyes, only thinner and smaller, and directed backward instead of forward. The sensilla of segment V is a little smaller, but still presents the same general features. In segment VI the organ is still eye-like, but its visual elements are fewer and the pigment cup imperfectly represented by loose pigment. In segment VII we find one or two visual cells and a little scattered pigment. In the following segments we usually find the

sensillæ in about the same condition. In no other species hitherto described do we find the sensillæ passing by such gradations into the principal eyes. *The serial homology of these organs with the eyes is, then, a fact demonstrated not only by the embryological development, but also by the structural gradations exhibited in the adult animal*" (pp. 391, 392).

The existence of a series of rudimentary sense-organs in Vertebrates was brought to light in 1885 by Froriep and Beard. While it is by no means clear that they are the homologues of the sensory papillæ of annelids, it is nevertheless probable. It is now generally admitted that they constitute the basis from which the higher sense-organs of Vertebrates are derived. These organs are arranged in longitudinal series. The series has become rudimentary or lost in the adult forms of higher existing Vertebrates, but they are present in the embryos. Kupffer and others have shown that there are at least two series of these organs, the upper one corresponding to the lateral line of comparative anatomy, and the lower one embracing the so-called branchial sense-organs of Beard. From the upper series the ears and nose are probably derived, and possibly the eyes.

Allis ('88), in his well-known memoir on the lateral line of Amia, gives figures that show a connection between the epithelium of the nasal pit and that of the lateral line. His Fig. 1, Pl. XXX, representing a larval form one day old, shows the nasal epithelium forming part of the lateral line. In subsequent growth it follows the same course that the surface organs of the lateral system do when they become canal organs. Allis says, p. 537: "The nasal pits are inclosed in the same way that the lateral canals are, and the short canal so formed is at first continuous with the canal inclosing organ 5 infra-orbital."

There is now general agreement that the ears belong to the lateral line series, but there has been much dissent on the part of some morphologists to admitting the eyes to the same category; but the grounds for the opposition seem to me to be largely removed. It must be said, moreover, that the organs of taste and touch have not as yet been shown to have any genetic relation to the ganglionic sense-organs.

I am glad to acknowledge my indebtedness to Minot's introductory remarks to his chapter on the Sense-Organs. He says: "Very suggestive in this connection are the observations of H. V. Wilson ('91), of a thickening of the nervous layer of the epidermis on either side of the head in the bass embryo (Serranus atrarius). This thickening forms a long, shallow furrow, which subsequently divides into three parts, of which the first becomes a sense-organ over the gill-cleft, the second, the auditory invagination, and the third, the Anlage of the sense-organs of the lateral line. This peculiar development confirms the notion that all these organs belong in one series, but the appearance of a continuous thickening as the Anlage of them all has, as yet, been observed only in this fish, and may not indicate a corresponding ancestral condition. Unfortunately, Wilson was unable to make out anything as to the connection of the sensory plate with the ganglia. The sense-organ above the gill-cleft, although differentiated, is a larval structure only, and disappears in the adult."

A quite similar condition is now known to obtain in some elasmobranch forms. Mitrophanow, in 1890, published a preliminary report of his observations on the lateral line of Acanthias and other Elasmobranchs. In 1893 he published a full report of the same, illustrated by many figures. He describes a continuous thickening of the epidermis along the sides of the head embracing the territory of the roots of the seventh to tenth nerves. From this thickening there is separated the material for the auditory saucer, the branchial sense-organs, and the beginning of the lateral line. My observations on this region in Squalus agree with those of Mitrophanow.

In looking over the literature on the sense-organs one cannot fail to be struck by the remarkable uniformity of the sense-cells in the different kinds of sense-organs. They are easily reducible to one type, and this, of course, favors the view that they have been derived from a common form.

I. The Lateral Eyes.

Squalus acanthias is an especially favorable form for observing the beginnings of the optic vesicles. They make their appearance in this animal at a very early stage, while the neural plate is broadly expanded, and before the neural folds have arched upwards in any part of the embryo. I should estimate their appearance in this animal to be as early as in any other animal in which they have been described. Previous to 1889 it had been current opinion that the optic vesicles appeared very early only in the class of Mammals. Bischoff, Kölliker, His, Van Beneden, Heape, and others had recorded their early appearance in that group ; but it was regarded as a precocious development for some reason confined to mammalian forms.

More careful observations on the earlier stages have shown that the optic vesicles customarily arise in different animals much earlier than was supposed. In 1889 Whitman called attention to the very early appearance of the optic vesicles in Necturus: "Its basis being discernible as a circular area — after treatment with osmic acid, followed by Merkel's fluid — long before the closure of the neural folds of the brain." Dural noted the early appearance in birds. In 1893 Eycleshymer gave notes upon their appearance and structure in Necturus and other Amphibia. His most noteworthy observations are upon Rana palustris, a form hitherto unstudied, in which he finds a remarkably early development of the primary optic vesicles. They are very evident from surface study in this form, on account of the distribution of pigment granules in them, just after the neural ridges have begun to form. Cross-sections in these early stages show a considerable amount of histological differentiation from the surrounding cells.

The early differentiation of the optic vesicles had not been recorded in any elasmobranch form until the publication of my preliminary papers in 1893 and 1894, where the optic vesicles are described and their serial relation to other structures on the cephalic plate is shown. Since then I have had opportunity to confirm my observations on Squalus, and to extend them to some other forms ; for instance, in Diemyctylus and

Amblystoma the optic vesicles are well developed in very early stages. In Torpedo ocellata they show fairly well, and in the chick they may be readily made out while the groove is widely open.

In Squalus acanthias the optic vesicles are the first rudiments of sense-organs to appear. Counting out the gastricular cavity (and the neural segments) they are the first organs of any kind to be established. Strangely enough, their early history in these animals has been entirely overlooked. Balfour's statement that "The eye does not present in its early development any very especial features of interest," seems to have withdrawn attention from that organ in the elasmobranch fishes.

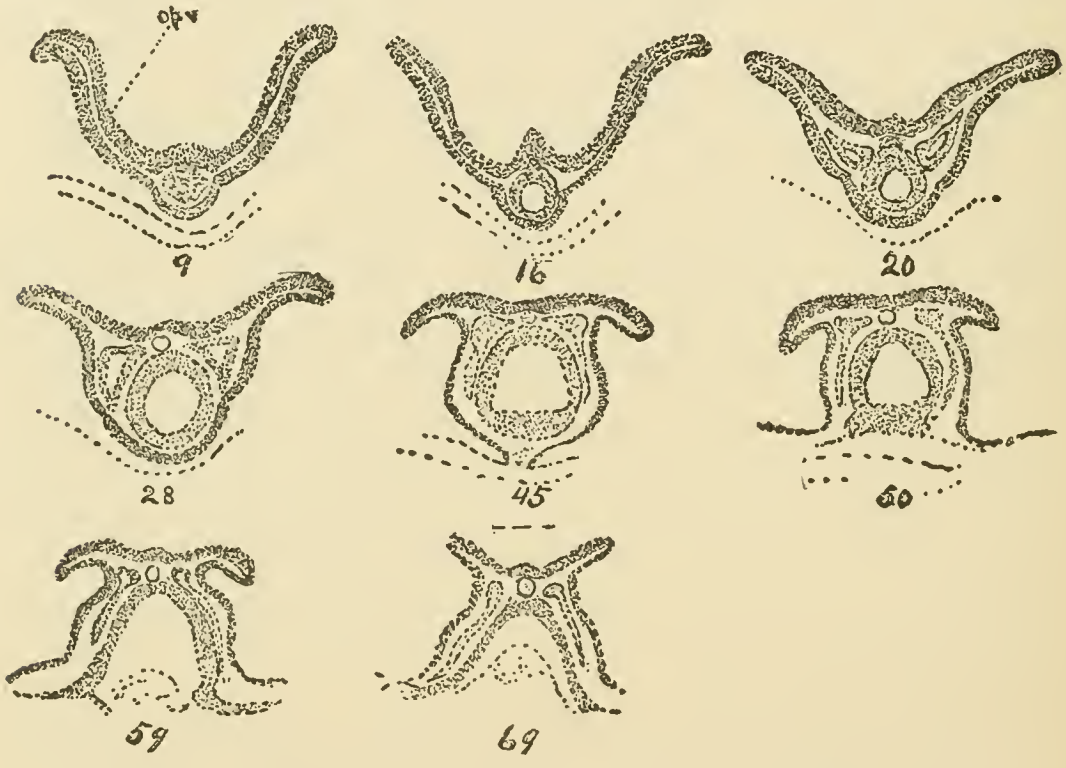
In order to fix clearly the time when the optic vesicles first appear in Squalus, we must glance over the early steps in the formation of the head-plate. Pl. XXVI, Figs. 1, 2, 3, represent three stages that immediately precede the formation of the eyes. In Fig. 1 the embryo is well established; it is a stage slightly older than Balfour's stage *B*. The front part of the embryo is not broader than the rest, and the curve of the anterior margin is uniform and unbroken. In Fig. 2 we note two changes, *viz.*, that the front end is broader than the part just behind it, which is apparently constricted, and the anterior margin is drawn out into a rounded median cusp. The head-end continues to expand laterally (Figs. 3 and 4) until we may (with some appropriateness) speak of it distinctively as a head-plate. In the meantime the trunk region has grown longer, and is relatively narrower, so that the entire axial embryo has a characteristic appearance of a narrow body terminated by a rounded plate-like head. When this stage has been reached the first satisfactory traces of the eyes become visible; when first formed they present the appearance that might be produced by pressing down lightly upon a plastic surface with two rounded dies. They are circular areas slightly concave upwards, and occupy a position far forwards on the cephalic plate. The early stage of these vesicles may easily escape observation, as they become evident only when the light strikes them properly, and when they are in a favorable position for the observer.

Fig. 5 is engraved from a photograph in which the optic vesicles show very well. The specimen from which the photograph was made showed about six mesoblastic somites, and was $2\frac{1}{10}$ mm. in length. The circular areas may be made out satisfactorily in slightly earlier stages, in which only three mesoblastic somites are differentiated, and in which the neural folds of both head and trunk are not only broadly expanded, but are even ventrally curved. The circular depressions are at first separated from one another by a raised welt. This has already been spoken of in Part I as a tongue-like process extending from the median-anterior tip backwards to two-thirds the length of the cephalic plate. It continues to be a prominent feature of the cephalic plate for some time. I can offer no suggestion as to its significance, outside of the obvious suspicion that it may represent a proboscis of some kind, or that it may be related to the large notochord of this region. On this point I have no conjectures to make. The depression to form the infundibulum starts a little after the first appearance of the optic vesicles, and cuts the median process in two, so that the anterior tip is separated from the rest, and the depression for optic vesicles and infundibulum combined reaches across the median plane of the cephalic plate (Figs. 6, 7, 8, 9, *etc.*). The optic vesicles, however, do not reach to the lateral margins of the neural folds ; the latter are much expanded beyond them.

It is evident that we cannot, in a strict sense, speak of such vesicles as "diverticula of the fore-brain." The "diverticula" are found before the fore-brain. The depressions once started grow deeper and press outward laterally ; when fully formed they are cupped, almost rounded in outline, concave from within, and they form rounded elevations where they come in contact with the outer layer of epiblast. Figs. 6, 7, 8, 9, 10, show very well the appearance presented by these primary optic vesicles when viewed from above. Fig. 7 is magnified higher than the others, and is, therefore, larger, but the others are all uniformly enlarged. These figures all show the interior of the optic pits. They represent a range of stages only slightly older than that shown in Fig. 5. In all of them the depression in which the optic vesicle lies extended across the median

plane, and in its median portion, the depression develops into the infundibulum.

The lateral depressions, which form the optic vesicle, sink downwards more rapidly than the median part of the depression, and as a result there is left a ridge or keel separating the optic vesicle and connecting the anterior-median tip of the head-plate with the central tongue-like process. Miss Platt, in her studies on the head of this animal, says: "It may be here

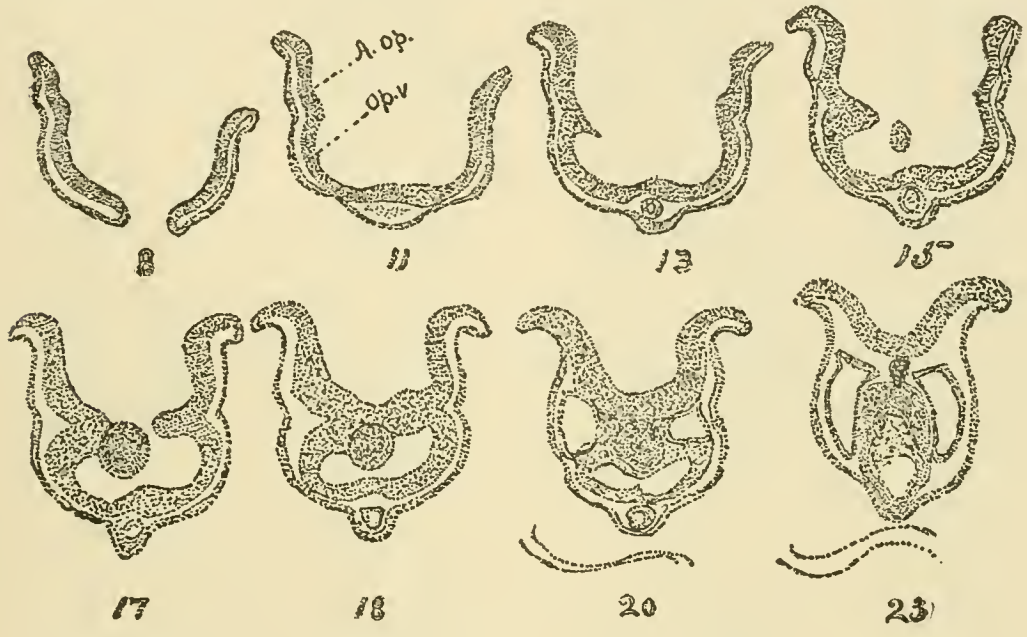


CUT 8. — Eight transverse sections of the embryo shown in Fig. 8, Pl. XXVI. × about 30 diameters. The numbers below the sections refer to their position in the series.

noticed that the downward growth in the floor of the brain, which gives rise to the infundibulum, is primitively bilateral, and not median," and I think, therefore, that she saw these circular optic pits in an early stage, but interpreted them as lateral pockets of the infundibulum. There might be reason to doubt whether they are the optic vesicles, if they could not be traced with perfect continuity into the eyes, and if there were not evidence of histological differentiation in their areas.

By the time the neural folds of the head begin to rise the optic vesicles are very prominent, and may be observed from the outside and from within. Fig. 13 shows an embryo viewed

obliquely from the left side, and the optic vesicle of that side is seen from the external surface, where it presents the appearance of a rounded eminence. On the opposite side the optic vesicle is seen from within. Cut 9 shows transverse sections of the head of the same embryo. Figs. 16 and 17 show embryos in which the optic vesicle (*op. v*) shows from without, and also from within (*op. v*). In Figs. 19 and 22 the optic vesicle shows clearly from the outside. Transverse sections of the

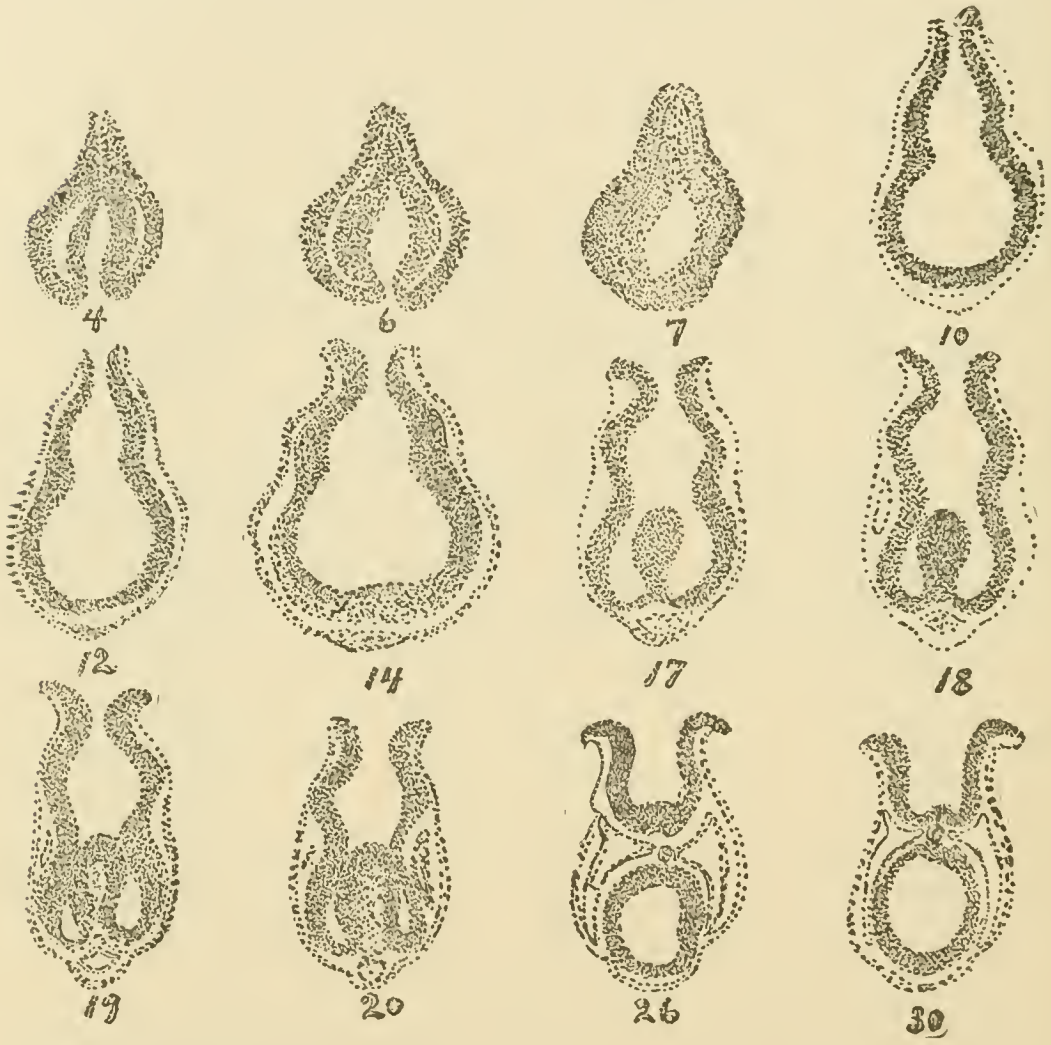


Cut 9.— Eight transverse sections of the embryo shown in Pl. XXVI, Fig. 13. × about 30 diameters. The accessory optic vesicles (*A. op.*) have been formed.

embryo photographed in Fig. 23, after partial closure of the neural groove, are illustrated in cut 10.

These optic vesicles are so well developed at an early period in Squalus, that it seemed to me probable that similar early formed vesicles might exist in Torpedo ocellata, and have been overlooked by the Zieglers, who have studied the early stages of that form in 1892 ; accordingly I procured embryos of Torpedo ocellata from the Zoölogical Station at Naples, and studied the head region with some care. The first thing noted was that the embryos of Torpedo ocellata are not nearly as favorable objects for observations as those of Squalus. They are smaller and the beginnings of sense-organs, branchiæ, and other structures about the head are by no means so clear as

they are in Squalus. As far as I am able to discern, there is no very distinct differentiation of the optic vesicles, in the early stages of this form; nevertheless, they are present at the stage designated *C*, by the Zieglers, and perhaps a little earlier. In studying the actual specimens, I had occasion to note that



CUT 10.—Twelve transverse sections of the embryo shown in Pl. XXVI, Fig. 23. × about 30 diameters. The numbers below the sections refer to their positions in the series. In Section 14 the optic vesicles show below and the depressions for the mid-brain vesicles above.

the Zieglers' models are admirable representations of the embryos of the form they are intended to represent. The optic vesicles are much fainter than they are in Squalus, but nevertheless are similar to them. I have studied them in Torpedo both in surface views and in sections. One noteworthy fact is that they have been sectioned and actually figured by Ziegler in his Pl. IV, Fig. 19[1]. It will be noted in this figure, that the brain-walls bulge outwards on either side and these depressions

mark the interior of the optic vesicles. Pl. XXIX, Fig. 65, is a drawing of one of my sections of Torpedo slightly younger than the one of Ziegler's just referred to. The optic vesicles are indicated at *op*.

The external appearance of the optic vesicles in later stages is shown in Pl. XXVII, Figs. 34, 35, 36, 37. When the stage represented in Fig. 35 has been reached the lens shows externally ; it is formed in the usual vertebrate way. The choroid fissure shows in Figs. 36 and 37. My studies have not been made to include the later stages of development of the optic vesicle, but are confined to its earliest differentiation.

Sections of the earliest-formed circular areas, show something in the direction of histological differentiation. Mitoses are more frequent and more of the cells are elongated and pear-shaped than in the other parts of the cephalic plate. The differentiation is by no means as marked as in the Rana palustris figured by Eycleshymer — nevertheless indications of change are not wholly lacking. My sections are too thick (10μ to 15μ) for satisfactory histological study, but one can see in them that the middle of the walls of the optic cups are areas of differentiation. The differentiation does not progress far until later, but the frequency of dividing cells, and their change of form continue, in these early stages, to be marks for distinguishing visual epithelium from that of the surrounding brain-walls. The dividing cells are neuroblasts, and these are known to be points from which the nerve-fibres spring. Their presence in any considerable number would, therefore, indicate a differentiation in the direction of increase of sensibility.

The eyes are the highest developed of the sense-organs, and in that particular stand at the head of the series. But, do the eyes belong to the same series with the other sense-organs, or do they occupy a position by themselves, can they be homologized with the rest ? This is a puzzling question, over which there has been much controversy, and upon which there is still much difference of opinion among anatomists. The stock objection to their being classed with the other sense-organs, is this : — they have apparently been derived from a different basis, and their nerves are developed in a different way. The

eyes are formed out of neural-plate material as diverticula of the fore-brain, while the other sense-organs arise outside the neural plate ; they have an independent epiblast origin.

It is questionable, whether any particular stress should be put upon that argument, as the neural plate is at best only a part of the modified epiblast ; moreover, the suggestion that the neural plate is, undoubtedly, very much widened and expanded from its ancestral condition, and that certain sensory area, originally lying outside, may have been included in it, does much to offset that objection. The neural plate has been a prodigiously long time in forming, and the eyes have been brought into closer relationship with it. The plate is broadest in front, and the eyes are the most anterior sense-organs, and have become included in this expansion. While there is not sufficient data to give a wholly satisfactory answer to the question propounded, the assumption that the eyes are closely related to all the others is not without foundation, and we are now in the attitude of awaiting further facts.

II. Accessory Optic Vesicles.

The neural plate, while still in very young stages, and while the neural grooves are widely open, becomes the seat of some accessory differentiations that resemble the optic vesicles. So far as I know no observations upon these structures have been recorded except those in my preliminary account in the Journal of Morphology. I quote from that paper : But, more interesting than the fact of their (the optic vesicles') very early appearance in Elasmobranchs, is their apparent relationship to other depressions that are formed upon the cephalic plate, behind the already established optic vesicles. The new involutions referred to, make their appearance upon the cephalic plate just back of the optic vesicle. Two of them (Fig. 13, *ac. v* and Fig. 17, *ac. v*^{1}, *ac. v*^{2}) take precedence of all others in development, and are so distinctly formed as to afford a good basis for comparison with the optic vesicles. They are circular depressions formed in front of the latter, and they produce upon the exterior corresponding rounded elevations.

The optic vesicles are formed first, and when, at a very little later stage, the others arise behind them, it appears as if the process of eye-formation were repeating itself serially.

These circular pockets not only arise in a similar way, but structurally resemble the optic vesicles. In cross and longitudinal sections the cells of the sunken patches are similar to those in the eye pockets. They may be designated *accessory optic vesicles*.

The assumption that they are primitively visual in character may be going too far, but the basis upon which it rests will be shown directly. The least that can be said of them (from their structure, the place and manner of their appearance), I think, is that they are segmental sensory patches.

These structures begin to appear soon after the eye vesicles. There are several pairs of them stretching in rows back of the eyes, as stated above, the two anterior pair are the most prominent. They gradually grow fainter; the anterior one is the best differentiated; the second one is not so clear, and the succeeding ones grow fainter as we pass backwards. I have counted as many as four pairs in surface study; but sections show that the series is more extensive and contains not less than eight pairs of these circular pits. The two anterior ones are well shown in Figs. 9 and 10. These are the earliest surface indications. In Fig. 17, which is slightly more advanced, the accessory structures are clearly developed; the figure shows four pairs behind the eyes. In longitudinal sections of the same specimen, Pl. XXIX, Figs. 76–87, eight pairs may be counted. The form of the depression, as seen at first from the surface view, is ovate; they soon become circular patches, and form themselves into concave, shallow cups. I have seen them in many specimens.

It will be noted that they make their first appearance while the neural plate is broadly expanded, and spring into prominence while the neural folds are growing upwards. It is during the rise of the neural folds that similar, but fainter, vesicles can be made out, in surface study, behind the two anterior pairs.

Pl. XXX, Figs. 88 to 112 show twenty-five transverse sections of an embryo very slightly older than that represented in

Pl. XXVI, Fig. 16. The sections 88 to 100 lie in the region of the cephalic plate. The following sections, 105 to 112, lie in the neck and trunk region. These sections are remarkable in bringing to light serial depressions along the walls of the neural folds. They show that the serial cup-like differentiations extend back of the cephalic plate. I have not been able to determine the number of serial differentiations of this character in the embryo, but it is clear that there are several pairs behind the cephalic plate upon which I have noted, in surface study, four pairs in addition to the primary optic vesicles.

My sections are not favorable for a critical study of the histological conditions, but it is clear, from them, that a differentiation starts in the anterior patches similar to that mentioned above, for the true eye vesicles. There is a greater frequency of dividing cells and many of the cells become elongated and pear-shaped.

Comparisons of the two anterior pairs with the eye vesicles give the following points of resemblance. They are formed in precisely the same way, and present the same general appearance; viewed from within they are all similar concave cups; they produce corresponding elevations on the outer surface, and, if observed from the outside, they form a series of rounded knobs serially arranged, the eye being the anterior terminations of the row. The marked differentiation in the eye takes place in later stages. There are, however, the histological features spoken of above that serve to distinguish it in early stages, and the accessory vesicle shows the same characteristics.

These resemblances, coupled with the existence of serial eyes in some of the Invertebrates, has led to the assumption that in Vertebrates these rudiments represent accessory optic vesicles. The fate of the anterior pair would also favor this view; they pass into the pineal sense-organ, a structure whose visual character is acknowledged.

If the view expressed above is true, we have a multiple-eyed condition in the embryos of these animals. This is common enough in Invertebrates, but has not been previously noticed in Vertebrates.

These accessory structures are present for a brief time only. That region of the head, behind the optic vesicles and in front of the ears, becomes the seat of great modifications, and, as the neural groove closes, the structures described give way to later formed ones. They are, therefore, not only embryonic structures, but are also transitory. It is a truism in developmental history that the more primitive characteristics appear first, and the secondary modifications come in later. The structures described are among the most primitive that have been preserved in this very ancient group of Vertebrates, and should be of significance in indicating the ancestral relations of the eye. I have spoken of the disappearance of the organs, but I have been able to trace the anterior pair further along, an account of which will be given in the next section on The Pineal Sense-Organs.

I have also traced out similar rudimentary organs in the embryo chick, at about the conventional 24-hour stage ; there is a series of seven vesicles behind the optic vesicles. These structures are rudimentary, and very quickly fade away.

All this acquires new interest when taken in connection with the researches of Whitman on the eyes of leeches. As indicated above, he showed that the eyes of the leeches are derived from segmental sensory papillæ. It now appears that we have traces of a somewhat similar line of segmental sensory organs in Vertebrates. It is essential to say *traces,* for these structures are transitory, and do not rise to a high grade of differentiation, except in the case of the pineal sense-organs. They may never, in the vertebrate group, have been raised to the rank of eyes, but it is certain that in Annelids similar segmental sensory patches have been so raised. It is, however, reasonable to suppose that members of this series have been functional, even in Vertebrates, when we recollect the highly developed condition of the pineal eye in some forms.

There is really very little direct evidence to support the proposition that the Vertebrates are derived from multiple-eyed ancestors, although it is a conception that has been in the minds of morphologists for some time. Besides the indications of such a condition afforded by the facts of this paper there

has recently come more evidence bearing in the same direction. This consists in the discovery of multiple pineal eyes, in several distinct forms, and, in one case, the existence of three distinct nerves to the pineal organs (see section on The Pineal Sense-Organs).

Whitman, in his paper just cited, says: "Although the evidence appears to me conclusive that the eyes and the segmental papillæ were, originally, morphological as well as physiological equivalents, it does not, of course, follow necessarily that both organs now have the same functional significance. The original papillæ may have represented sense-organs of a more or less indifferent order, among which, in the course of the historical development of the leech, a division of labor was introduced, a few at the anterior end becoming specialized as light-perceiving organs, the rest either remaining in their early indifferent condition or becoming specialized in some other direction.

"The discovery that these papillæ are sense-organs might lead us to speculate on affinities of a distant and somewhat uncertain nature, such as are supposed by the writer, in common with many others, to exist between annelid worms and Vertebrates. At all events, the existence of such organs in the leech furnishes a broader basis for the discussion of the question whether the Vertebrates and Annelids have been derived from a common form possessing metameric sense-organs, as was first argued by Dr. Eisig of the Naples Station. Assuming that the sense-organs of the Vertebrate and the segmental papillæ of the leech may be traced to a common origin in some remote ancestral form, it does not follow that they should now present close structural resemblances. It is far more important to show that they possess certain general features in common. The most important of their common features is undoubtedly their metameric origin. The nerve-supply forms another feature of fundamental importance, in which, according to the interesting observations of Mr. Beard, on the segmental sense-organs of the lateral line (*Zool. Anz.*, VII, Nos. 161 and 162) of the Vertebrate there is essential agreement. The developmental history of these

lateral organs in the fish, where they make their first appearance as *segmental papillæ* in the strictest sense of these words, cannot be explained on a more satisfactory hypothesis."

III. The Pineal Sense-Organs.

1. *Growth of Knowledge regarding the Pineal Sense-Organs.*

The pineal outgrowth has attracted much attention since the discovery, in 1886, of its eye-like structure. Since then it has been accepted by morphologists as a rudimentary sense-organ. The earlier literature bearing on the subject has been thoroughly reported on by Spencer ('86) and Francotte ('89). Several recently published papers taken together put the subject in a new light, and, as Ritter ('94) has said, "At no time since the epiphysis and pineal eye have been the topic of investigation has it been a more interesting or a more inviting one than it is just now."

The growth of our knowledge regarding this remarkable sense-organ, scattered through the various publications of Spencer, Béraneck, Francotte, Strahl and Martin, Leydig, and others, may be reduced to the following summary :

First came the demonstration of its eye-like structure in Amphibia, by De Graaf, and in Lacertilia by Spencer. De Graaf's publication is much briefer, and was published only a few months before Spencer's extensive study. Both authors investigated the structure in adult forms. Spencer studied its condition in twenty-eight different species of Lacertilia, and demonstrated very clearly that even in the adult forms of these animals, this organ has an eye-like structure, with a lens, a pigmented retinal layer, and a nerve. It seems doubtful, in the light of recent work, whether the fibrous strand connecting the eye-capsule with the epiphysial stalk, and designated the nerve by Spencer, is really nerve or not.

Following the study of the adult structure came investigations of the embryonic conditions. The first studies dealing with the embryonic development of this organ, and with its

minute structure in the early stages, were those of Strahl and Martin. Along this same line were the studies of Béraneck, Francotte, Leydig, and others. By these investigators the organ was traced backwards to a certain point in its history, that is, to the time when it springs from the roof of the thalamencephalon as a tubular outgrowth, like the tip of a finger of a glove. To this period also belongs the discovery of a nerve of transitory existence. The investigations were confined almost entirely to two groups of animals, Amphibia and Reptilia. In the embryos of these animals many points of minute structure were worked out, such as the distribution of the nerve within the eye-like capsule, the division of the retinal part into distinct layers, the fact that the pineal eye is higher developed in the embryonic periods than later, etc.

The next step was the discovery that there is more than one epiphysial outgrowth. Selenka, Leydig, Eycleshymer, Hill, successively called attention to the existence of two distinct outgrowths, or vesicles, from the roof of the thalamencephalon in Anguis, Amblystoma, and Teleosts.

Hill's studies on these two vesicles are the most complete. He has shown the existence in Teleosts and Amia of two vesicles, anterior and posterior, and has traced them through the stages of development. The posterior develops into the epiphysis and the anterior degenerates. The distal portion of the former is histologically very complex, and receives a nerve from the posterior commissure. Hill made out the distribution of nerve-fibres in this part of the epiphysis. He traced quite clearly the history of the vesicles.

Two vesicles, an upper and a lower, have long been known to exist in Petromyzon, in both embryos and adults. It is much to be regretted that we do not know their embryonic history, for much depends on this. The most recent paper on the epiphysial organs in Petromyzon — that of Studnička — does not clear up the question of the origin of the two vesicles. Histological material was lacking in just the stage required. The two outgrowths in embryos outside the Cyclostomes have been designated epiphysis, for the hinder, and pineal eye for the anterior. Studnička designates them pineal and parapineal,

respectively, in the Cyclostomes. As I shall show later, the so-called paraphysis is a different structure.

Béraneck showed that the pineal eye and epiphysis have been confused, and, on account of the existence of the nerve from outside sources, he argued for the complete independence of the pineal eye.

Klinckowström has placed himself in opposition to this opinion, showing the eye vesicle is formed as an outgrowth from the epiphysis.

Next to the discovery of two or more epiphysial outgrowths may be mentioned the existence of accessory pineal eyes. The earliest publication containing an account of such structures is that of Duval and Kalt, in 1889. In a brief note (Des Yeux Pinéaux Multiples chez l'Orvet) these authors record the finding of two or three accessory pineal vesicles in Anguis. They do not say whether adult or embryonic forms were studied.

Carrière ('89), in the same year, noted the occurrence in embryos of Anguis of a very rudimentary accessory pineal vesicle.

Leydig ('90) observed these structures independently in embryos of the same animals. He showed that there is much variability, both as to the presence and the histological structure of these accessory capsules. In some individuals they are lacking, and in others of the same age present and well developed. He records the occurrence, in some cases, of two accessory organs, a larger and a smaller one. The larger one resembles the pineal eye in structure and arrangement of pigment. The smaller one is very rudimentary. Leydig compared them to the ocelli of insects. It is clear from his account that variability and inconstancy are characteristics of these accessory organs.

Prénant, in the latter part of 1893, again records the occurrence of one accessory pineal eye in the embryos of Anguis. He directs attention to the fact that up to that time they had been discovered only in a single species, *viz.*, Anguis fragilis, and further, that they were all in embryos, and not in adults. Ritter ('94) has recently recorded the occurrence of such an accessory organ in the adult Phrynosoma.

The above observations, taken together, give sufficient positive data to establish the fact that there appears, from time to time, in some animals the vestiges of accessory pineal organs, and that they are variable and inconstant in their occurrence.

The most recent advances consist in carrying the history of these organs backwards, and showing them to be connected with patches of sensory epithelium that arise on the cephalic plate in very young stages, and in the discovery, in Iguana, of three distinct nerves connected with the epiphysial outgrowths. The former work was done by the present writer, and the latter by Klinckowström.

As indicated in the preceding section, there are several pairs of cups on the cephalic plate back of the optic vesicle, formed while the neural groove is widely open. I have designated them accessory optic vesicles. In the process of closure of the neural groove the anterior pair are brought together, and form part of the thalamencephalon, from the roof of which the pineal outgrowth is derived. This process will be described more in detail.

Klinckowström ('93) has recently shown in Iguana the presence of two nerves connected with the anterior outgrowth or pineal eye, and sometimes a third connected with the posterior outgrowth or epiphysis.

These observations suggest new interpretations; still it is not an auspicious moment to indulge in speculation. It is clearly evident that we need more data regarding these organs and their relationships. But the trend of these discoveries is to strengthen the suggestion already made in this paper, that, primitively, the Vertebrates were multiple-eyed. The presence of accessory pineal eyes, the discovery of serial sensory areas on the cephalic plate from which epiphysial outgrowths arise, and the presence of two or three pineal nerves, are all consistent with this interpretation. So far as the evidence goes, there is more than one epiphysial outgrowth, and therefore I have headed this section, The Pineal Sense-Organs. In what way it will be necessary to qualify this proposition will depend on subsequent discoveries.

The results of all the embryological studies on the epiphysial outgrowth between 1887 and 1893 were to settle on one point of common agreement regarding its origin. Each investigator successively found it to arise as a tubular outgrowth from the roof of the thalamencephalon comparatively late in ontogeny, after the parts of the brain are established. No earlier trace of it had been found; but there was a previous undiscovered history, and it is now timely to make the inquiry, What is the very beginning of the pineal sense-organs?

2. *Remote Origin of the Pineal Outgrowth.*

As already indicated, there are upon the cephalic plate accessory eye vesicles which have made their appearance while the neural groove is widely open; and in tracing their fate I shall attempt to fill up that gap in the history of the pineal outgrowth from the time these cup-like structures appear upon the cephalic plate to the time the tubular outgrowth begins from the thalamencephalon.

As the walls of the neural groove grow upwards these cup-like structures are carried up with them; and there comes a time (Figs. 13, 31) when they may be seen from the outside as rounded eminences, and from the inside as concave cups. When the edges of the neural groove meet in the middle line the cups are approximated, and come to form part of the thalamencephalon.

While these changes have been going on, further differentiations have been taking place in the walls of the brain. The bulging of the walls to form the mid-brain vesicle has come on insidiously, and has taken a position behind the vesicles of the paired eyes, in apparently the same position previously occupied by the accessory vesicles. These transformations are confusing, as the walls of the mid-brain resemble the accessory optic vesicles grown larger. In an earlier published paper I made the mistake of identifying the mid-brain with the accessory optic vesicles; but it was merely an error of identification, and did not affect the main contention of that article.

In working out the details of the formation of pineal outgrowth in Squalus, it soon becomes apparent that the surface

contours of the head are considerably altered by the distribution of mesoblastic cells lying between the external layer of epiblast and the brain-walls. The mesoblast forms a pad of varying thickness in close contact with the brain-walls; the depressions are filled, and in some places thick patches of mesoblast give rise to external markings that render the surface appearances untrustworthy. There is, for instance, a deep pad of mesoblast filling up a depression at the sides of the cerebellum, and also extending forwards on to the mid-brain; at the external surface the pad assumes a circular form, and it is that pad of mesoblast which is seen in Fig. 32. The true mid-brain vesicle in that figure is the eminence marked *mb*. The accessory optic vesicles are present, but are not well marked externally. They are in front of the protuberance marked *mb*.

In order to get a view of the brain-walls I have found it necessary to remove the mesoblast and its coverings of epiblast, and thus to completely expose the brain-walls. The brain thus laid bare, enables one to see with complete satisfaction its different parts and their relation to one another. A very interesting relation comes to light through the dissections. Somewhere between the stage with an open neural groove (Fig. 31) and the completely closed groove (Fig. 33) the mid-brain vesicle insidiously takes the former position of the accessory optic vesicle while the latter is carried forward by the process of cranial flexure. During the formation of the mid-brain vesicle the two structures become incorporated into one faintly bilobed protuberance (Pl. XXVIII, Figs. 38 and 39). The anterior part is the accessory optic vesicle, and the posterior one the mid-brain. The separation soon becomes complete, and by the stage represented in Fig. 40 the two are completely separated; but owing to the arrangement of the mesoderm the accessory optic vesicle is rendered indistinguishable from the outside. When the mesoderm is entirely removed it may be seen. All this takes place in very brief intervals of time, and a complete series of embryos representing the different phases of the closure of the neural groove is required to see it all. I must say also that the changes are so perplexing

that there exists in my mind some doubt as to the adequacy of the above account. It may have to be modified, but I have given the facts as they now appear to me.

Figs. 38–54 show a series of embryos that have been partly dissected in the way just indicated. They show characteristic changes in the brain-walls as seen from the exterior.

In Fig. 38 the walls of the neural groove have not yet met in any part of their course, and the thalamencephalon cannot be distinguished from the rest of the brain. The first pair of accessory vesicles are visible; they soon become incorporated in the walls of the thalamencephalon. I have not been able to determine whether it is the epithelium of the first pair of accessory vesicles only, or whether epithelium from the second pair is also included. Either the epithelium of the two pairs is incorporated into the walls of the thalamencephalon by being carried together, or the epithelium of the second pair fades into the surrounding substance of the brain-wall, which almost immediately grows into the vesicle of the mid-brain. Although I am doubtful as to whether the second pair of accessory vesicles pass into the inter-brain, I am sure that the epithelium of the anterior pair is so included.

Fig. 39 shows the first accessory optic vesicle and the mid-brain vesicle forming a common protuberance, but they are very quickly separated, and the anterior vesicle goes into the thalamencephalon.

In Fig. 40, which is somewhat older, this has occurred. The lateral walls of the thalamencephalon now consist of two shallow cups, approximated so that the structure looks lenticular when viewed from above. As a usual thing, the thalamencephalon is not evident at this stage before the removal of the overlying tissues. Fig. 41 shows, however, a specimen in which it could be seen before any dissection. Compare this with Fig. 32, which is the more usual appearance of an embryo of this age.

Fig. 42 is a dissection of the embryo shown in Fig. 41. The mid-brain, which, from external view, appears like a single rounded eminence, is shown after exposure to be bilobed.

Figs. 43, 44, and 45, which are successively older, show an increase in the size of the thalamencephalon, and marked changes in the mid-brain.

In Figs. 46 and 47 a faint furrow has appeared in front that serves to mark the boundary between the thalamencephalon proper and the cerebral lobes. This furrow is clearly defined in Figs. 48 and 49, so that we have now the thalamencephalon distinctly marked off from the rest of the fore-brain.

In Fig. 48 the optic vesicle and all the mesoblast have been removed, and the view is taken from the right side. The brain-walls show very clearly. The thalamencephalon is now bounded anteriorly and posteriorly by furrows. The two furrows run nearly parallel to one another, and they serve to mark off the inter-brain very distinctly.

The roof of the thalamencephalon is at this stage raised into two rounded eminences of nearly equal dimensions, an anterior and a posterior one. The posterior is, from the beginning, somewhat in advance of the other. It becomes the pineal outgrowth, while the posterior eminence goes on developing. The anterior one becomes greatly reduced by compression from the rapidly growing adjacent brain-walls. It very soon loses its rounded character, and becomes pressed into a semicircular fold lying in front of the epiphysis. It is, I think, equivalent to the Zirbelpolster of German authors. If longitudinal sections be made at the stage represented in Fig. 48, the two elevations show as in Minot's Fig. 319, p. 572, of his *Human Embryology*. In that figure they look like two equal vesicles springing from a single elevation of the brain roof, and opening by a common wide passage into the brain vesicle. No especial significance, I think, is to be attached to the fact that there are at first two equal embossments from the roof of the thalamencephalon. The anterior one is converted into a fold of the roof of the 'tween-brain that has long been recognized in different animals. It is designated Zirbelpolster by Burckhardt. It does not in any sense correspond to the anterior epiphysial vesicle discovered by Hill in Teleosts, but rather to the fold of 'tween-brain that lies in front of both epiphyses. Studnička has, however, marked the fold in diagrammatic figures of Tele-

osts, the anterior vesicle of Hill, but this identification is not right.

We have in this specimen the first appearance of the tubular outgrowth, which, according to previous authors, marks the beginning of the epiphysis. It is somewhat important to fix the stage at which this outgrowth begins. Its very first appearance as an elevation of the roof of the thalamencephalon is in the stage represented in Fig. 36 after the saucer-stage of the ear is past.

In Fig. 49 the posterior protuberance of the roof of the thalamencephalon is assuming a tubular form. It becomes the epiphysis. The surface of the mid-brain is now considerably changed. Two furrows have made their appearance, which divide the lateral walls into three nearly equal lobes. These do not show at all from the exterior before the removal of the epidermis and the mesoblast.

The gradual reduction of the thalamencephalon by compression between the central hemispheres and the mid-brain is shown consecutively in Figs. 53, 54, 55, 57, and 60. In Fig. 54, and later, the epiphysis appears pear-shaped from the side, but from in front the enlargement on the distal end is seen to be broader than thick. It is borne upon a hollow stalk.

Fig. 57 represents the brain of an embryo considerably older than in Fig. 56. The thalamencephalon is now very much compressed. The anterior half of it, which originally had a rounded protuberance growing from its roof, is now reduced to a small semicircular fold in front of the epiphysis.

Fig. 59 is a view upon the epiphysis of the same embryo from directly in front. It was obtained by removing the cerebral lobes, which lie in front of, and partly hide the epiphysis. The epiphysis is seen to be composed of a stalk and an enlarged distal extremity ; leading from the stalk on either side are the epiphysial peduncles. In this figure we are looking through the ventricle of the thalamencephalon into that of the mid-brain. On the inside of the lateral walls of the opening are two enlargements, the beginnings of the thalami optici. These contain the ganglia hebenulæ.

In this specimen the paraphysis had made its appearance as an outgrowth from the cerebrum. It is relatively late in arising, and is entirely distinct from any outgrowth from the thalamencephalon. The lateral walls of the mid-brain are no longer lobed as in earlier stages. Fig. 58 is the same brain viewed from above; we are looking directly into the cavity of the fourth ventricle.

Fig. 60 shows the brain of a still older embryo. It is the largest embryo of Squalus in my collection, measuring 35 mm. in length. The cerebral lobes are divided into right and left halves by a shallow furrow. From the back of the cerebrum rises the paraphysis (*p*); just behind this is seen the semicircular fold, belonging to the 'tween-brain, and behind that is the epiphysis, rising above and resting upon the anterior wall of the mid-brain. Fig. 61 shows a front view of the cerebral lobes after being removed from the rest of the brain; the paraphysis is also brought into view. Fig. 62 is the same brain with the cerebral lobes removed, and placed so as to give a full-face view on the epiphysis; the stalk is considerably longer than in Fig. 59.

I have not had material to work out the subsequent history of the epiphysis. In specimens about six inches long it is situated in the wall of the cranium, directly over the cerebral lobes, and is connected to the roof of the 'tween-brain by a long thread-like stalk. The enlarged distal end is about the same size as in Fig. 62. Cattie shows it in the adult with several capsules on the end of the stalk.

Sections of the stages just studied serve to confirm the observations made from the outside. They do not add very much to its features, speaking strictly morphologically. The surface views have been prepared in such a way that they may stand for reconstruction, but they are more accurate than any actual reconstruction can be, as they show the relations entirely undisturbed.

Figs. 119–124 show a series of sagittal sections covering the period of the first appearance of the epiphysis, the reduction of the anterior part of the thalamencephalon, and the beginning of the paraphysis and the choroid plexus.

Fig. 119 is a specimen of the same age as that shown in Fig. 48. It shows that the roof of the thalamencephalon is raised into two nearly equal protuberances.

Fig. 120 shows the beginning of the reduction of the anterior of these elevations, and the increased growth of the posterior one. In Fig. 121 the posterior elevation, or epiphysis, has become a tubular outgrowth, while the anterior one is reduced and depressed.

In Fig. 122 the tubular stalk of the epiphysis is considerably elongated, while that part of the roof of the thalamencephalon in front of it has become reduced to a narrow fold that has been carried ventrad. The paraphysis has now arisen from the roof of the prosencephalon. The beginning of the choroid plexus extends from the structure down into the ventricle of the fore-brain, and its posterior part is connected with the fold in front of the epiphysis. The anterior and posterior commissures are now distinguishable.

Figs. 123 and 124 exhibit substantially the same relations; the choroid plexus has considerably increased in extent, and is rapidly becoming much convoluted; the brain vesicles have become reduced. In Fig. 124 the distal end of the epiphysis is inserted into a cavity in the roof of the cranial wall, that is, the mesenchyme forms a cup over its rounded end.

3. *Comparisons between Epiphysial Outgrowths.*

The recent studies have done much towards establishing a basis for comparison between the epiphysial outgrowths in different groups of animals. Béraneck, Francotte, and others claim that the anterior epiphysial vesicle — commonly designated the pineal eye — is formed independently, having no generic connection with the posterior one, or epiphysis. Hill's observations on the Teleosts coincide with this view. Klinckowström, on the other hand, claims that the anterior one is formed at the expense of the posterior one, and substantiates his conclusions with figures of sections of Iguana, showing the two vesicles in union with one another. It is not difficult to conceive how the condition represented by Klinc-

kowström might be brought about, and still not be inharmonious with the other observations. The two vesicles might, in earlier stages than he represents, have been formed independently, side by side, and thrown into communication by an upward growth of the brain roof involving them both. Or, indeed, there may be variations in the details of the formations of these two vesicles.

However this may be, the results of the different observers all go to show conclusively that there are two (not counting the paraphysis) distinct outgrowths from the roof of the thalamencephalon of Petromyzon, Teleosts, and Lacertilia. The fate of the anterior vesicle varies in the different groups, while the posterior one persists in all as the epiphysis. The anterior one may be formed as a rudimentary organ of transitory existence, as in Teleosts; or it may be developed into the pineal eye, in front of the epiphysis, as in Lacertilia.

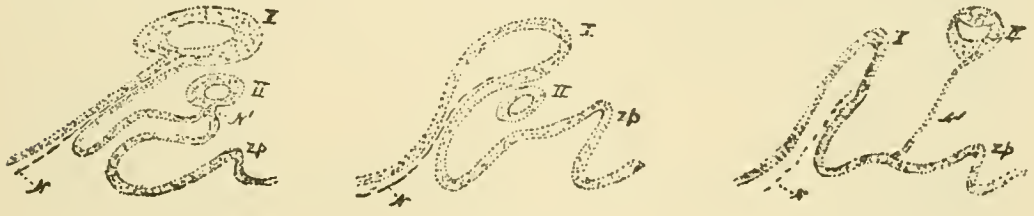
The sources of nerve-supply to the two vesicles have been made known in Cyclostomes, Teleosts, and Lacertilia, and these facts are important in establishing homologies. It has been shown (Studnička, Gaskell, Klinckowström) that the upper capsule of Petromyzon receives its nerves from the posterior commissure, and that the lower vesicle is innervated from the left ganglion hebenula. Hill, in embryos of Teleosts, has traced a nerve from the posterior commissure into the epiphysis and has studied its distribution in detail in that structure. The anterior vesicle of Teleosts disappears before it has formed any nerve connection with the brain.

Klinckowström ('93) has observed very interesting conditions in embryos of Iguana. In this animal he found in one case three nerves distributed to the parieto-pineal organs: one coming from behind and entering the epiphysis, and two coming from right and left ganglion hebenula, respectively, and entering the pineal eye. He found the right and left nerves in three different embryos of Iguana eighteen days old. In one case the nerve from the left ganglion hebenula was nearly the same size as that from the right, but in the other two individuals the left nerves were much reduced. It should be added that Klinckowström has found the nerve to the pineal

eye in this animal to come normally from the right ganglion hebenula, while in Petromyzon it comes from the ganglion of the left side. But, his discovery of the occasional presence of two nerves in Iguana, the left one being smaller, affords an interesting transition between the two. It also enables us to account for the marked asymmetry in the ganglia hebenulæ in Petromyzon, on the hypothesis that the right ganglion, in Cyclostomes, has, for some reason, ceased to have any nerve connection with the pineal organ, while the left ganglion has retained its connection.

As Béraneck has shown, a nerve leading to the pineal eye arises in front of the epiphysis, and has but a transitory existence.

Putting the facts together and basing relationships on the nerve-supply, and comparative study of the vesicles, we may express the probable homologies in the following diagrams and table:



PETROMYZON. TELEOSTS. LACERTILIA.

CUT 11.

I. Upper vesicle in Petromyzon, epiphysis in Teleost and Lacertilia.
II. Lower vesicle in Petromyzon, Hill's anterior vesicle in Teleosts, pineal eye in Lacertilia.
N. Nerve to posterior commissure. N^1. Nerve from lower vesicle in Petromyzon to Ganglion hebenula, embryonic nerve of transitory existence in Lacertilia.
Z.P. "Zirbelpolster."

		PETROMYZON.	TELEOSTS.	LACERTILIA.
Posterior epiphysial vesicle of Hill. Pineal organ of Studnička. Epiphysis of others.	I.	Is the superior vesicle. Nerve-supply from posterior commissure.	Is the epiphysis with nerve connecting it to posterior commissure.	Is the epiphysis nerve-supply from posterior commissure.
Anterior epiphysial vesicle of Hill. Parapineal organ of Studnička.	II.	Is the inferior vesicle.	Is formed as in the two other groups, but aborts.	Becomes pineal eye supplied with a nerve which early degenerates.

If these comparisons are well taken, we have in the Petromyzon, the epiphysis in its highest state of development. Its distal end is enlarged into an eye-like capsule that is more differentiated than their inferior vesicle which represents the pineal eye. This is the reverse of what is true in most Lacertilia, and, on this account the upper capsule of Petromyzon has usually been compared with the pineal eye of Reptilia and Amphibia. But the fact that the superior capsule in Petromyzon receives its nerve-supply from the posterior commissure, corresponding in this particular with the epiphysis of other animals, has much more weight in determining its homologies than any purely structural resemblances. We are on a better foundation, therefore, in comparing the upper capsule of Cyclostomes to the epiphysis of other animals, than in taking the other position. It may be noted, in passing, that the superior capsule is in the same relative position as the epiphysis, and the inferior one corresponds in position with the pineal eye.

This view would make the epiphysis eye-like in character, and there are many facts that weigh in that direction. Hill's studies speak strongly for the visual nature of the epiphysis. He shows a high degree of differentiation in the nerve cells to which the nerve from the posterior commissure is distributed. There is also formed in Iguana, Plica, and Phrynosoma an eye-like enlargement at the distal extremity of the epiphysis.

Between the condition in Petromyzon and that in the Lacertilia many gradations have already been brought to light.

In Teleosts, the anterior vesicle arises, as in the other groups, but it is transitory, and soon degenerates. It would appear from a recent paper by Ritter, that the anterior vesicle in Phrynosoma is more persistent than in Teleosts, but does not reach the grade of differentiation exhibited in the Lacertilia. He gives a figure showing the rare occurrence of the anterior vesicle in an adult Phrynosoma, and although large this capsule is not so highly differentiated as the capsule behind it, but to compensate for this the distal end of the epiphysis is highly developed. In other forms of Lacertilia,

the anterior vesicle reaches a high grade of perfection, while the epiphysis is not well developed. In most cases, however, there is a deposit of pigment in the distal position of the epiphysis. The nerve distribution in that organ is known only in the Teleosts.

We might carry our comparisons further and inquire whether the structure present in Selachians is to be homologized with the epiphysis or the pineal eye of other forms. The nerve relations are not known in the Selachians and, therefore, we have not, as yet, a satisfactory basis for comparison. In its structure and persistent attachment to the brain roof by a stalk, the outgrowth in Selachians resembles the epiphysis and one would be inclined to say that only the epiphysis is represented in the Elasmobranchs, and that the pineal eye is lacking. Klinckowström has offered the ingenious suggestion that in Selachians and birds the second or anterior epiphysis is never fully separated from the posterior, and this is worth thinking about. There is no positive evidence to support it.

However the above question may be settled, it seems to me that the fundamental features of the morphology of the epiphysis are tolerably clear, and that future work on this subject will be mainly in the direction of amplification and discovery of details.

The invertebrate homologies of the pineal eye are not known. Spencer supposed it to be homologous with the median eye of Tunicates, but that relation is a strained one.

Various authors have compared the pineal outgrowths to the ocelli of Arthropods. Leydig compares them to the stemmata of Insects, and shows that there are many resemblances to support the comparison.

Patten has recently studied the development of the eyes of Limulus, and argues for a homology between the median eyes of these Arthropods and the pineal eye of Vertebrates. He shows the median eye of Limulus to arise by the fusion of paired eye-stalks, giving us a case of undoubted union of originally paired sense-organs. I have claimed a similar occurrence in the epiphysis of Squalus. But it seems to me that the

evidence is too circumstantial at present to bear the interpretation that we already know the invertebrate homologue of the vertebrate pineal organ.

4. *The Double Nature of the Epiphysis*

receives support from two sources. It is difficult to interpret Klinckowström's discovery of a double nerve in Iguana on any other hypothesis. That interpretation is also strengthened by the claim I have made of tracing the epiphysis in Selachians back to a pair of epithelial cups on the cephalic plate.

Hill ('94) regards the two vesicles he has discovered in Teleosts as having been primitively side by side ; and Ritter ('94) has recently suggested that the epiphysis and the parapineal organ of Studnička are right and left mates rather than independent eyes of double origin. It will be remembered, in this connection, that Klinckowström found three nerves in one individual of Iguana, two from the ganglia hebenulæ, and one from the posterior commissure ; that Leydig discovered two accessory pineal organs in Anguis ; Duval and Kalt found as many as three of these organs, and I have found several pairs of epithelial patches back of the eyes on the cephalic plate, and have traced one pair into the epiphysis. It seems to me that all this evidence is more favorable to the idea that the pineal sense-organs are multiple, and individually of paired origin, than to the idea expressed by Ritter.

5. *Paraphysis.*

Considerable confusion has arisen in identifying the paraphysis in different forms. As soon as it was made known that there are two outgrowths from the roof of the fore-brain, it was at once assumed that the most anterior one is the paraphysis, and the posterior one the epiphysis. But there is now evidence that there are, at times, more than two outgrowths. Hill has shown in Amia the presence at one and the same time of three tubular outgrowths from the roof of the fore-brain. Two of these come from the thalamencephalon, and the anterior one arises from the prosencephalon. He points out that

the latter is the paraphysis proper, and that in Amia we have to deal with two other outgrowths from the roof of the thalamencephalon. These he makes homologous with the two vesicles he has described in Teleosts. Hill concludes that in the latter group the paraphysis is lacking on account of the non-development of the choroid plexus. This identification is in harmony with the original description of the paraphysis. Francotte, who described it in 1887, and Selenka ('90), to whom the credit of its discovery is usually accorded, both state that it arises from the prosencephalon. Selenka says: "Wie das Zwischenhirn seine Epiphyse, so hat das Vorderhirn seine Paraphyse." Francotte suggested that it represents the beginning of the choroid plexus.

Minot ('92), p. 690, designates the anterior vesicle discovered by Hill ('91) in Coregonus the "paraphysis," but the structure in question arises from the roof of the thalamencephalon, and between it and the prosencephalon is a marked downward fold in the brain-wall, which in many other forms is present behind the paraphysis. It is more probable, as Hill suggests in his later paper ('94), that the paraphysis is lacking in the Teleosts.

It is to be understood that in some forms there are two epiphysial outgrowths from the thalamencephalon that are entirely independent of the paraphysis. When the latter structure is present in conjunction with the former two, as in Amia, there are then three separate outgrowths from the roof of the fore-brain.

As already indicated in this paper, the paraphysis is present in Squalus as an outgrowth from the prosencephalon, and is connected with the choroid plexus.

IV. The Beginning of the Auditory Organ.

The formation of the auditory saucer is preceded by a thickening of the epidermis along the sides of the head in the auditory region. The thickening portion occupies a larger area than that used by the ear vesicle when it is first formed, and it is confluent with the epidermal thickening just above the

branchiae. As Mitrophanow has pointed out, this thickened patch of epithelium is composite in character ; it embraces not only the epithelium for the formation of the auditory plate, but that to form the so-called branchial sense-organs, and also the beginning of the lateral line. I had noted this relationship in Squalus before seeing Mitrophanow's paper, and I find no comparison that my results correspond — so far as the earlier stages are concerned — very closely with his. He has, however, carried his studies considerably farther, and has shown how the different branches of the lateral-line system arise.

The general relationship noted between the auditory plate, the branchial sense-organs, and the lateral line is very similar to what Wilson found in sea bass. In that form the connection between ear, branchial organs, and lateral line is evidently more clear on account of the presence of a distinct sensory furrow and the way in which the separation into three parts takes place.

The general thickening in the sharks is not so well circumscribed. The fact that these different organs proceed from a common epithelial thickening indicates relationship of a fundamental kind. After separation the ear gives further evidence of its relationship with the lateral organs by preserving its canal (endolymphatic duct) connection with the exterior and developing in a manner that is characteristic of the canal organs of the lateral line.

Mitraphanow departs from the usual point of view that the organs of the lateral line are metameric, and in that particular, I think, I should be inclined to follow him.

The auditory plate is at first differentiated from the general thickening, and its epithelium then becomes gradually rounded up into a circular area, that is depressed in the centre — the auditory saucer. This structure sometimes covers the space of three neuromeres, and I have one surface preparation in which the outer epithelium shows three bars running vertically across it; these bars correspond with the underlying neuromeres.

We may interpret these superficial bars either as being moulded upon the underlying neural segments, or as being

due to the same general cause as the neural segments. I am of the opinion that the latter alternative is the correct one.

When first formed, the auditory saucer is opposite the ninth neuromere; but subsequently, while it is still in the saucer condition, it shifts backwards, and comes to lie at the side of the tenth neural segment, and later still, as a capsule, it lies opposite the eleventh neuromere.

The history of the auditory vesicle in sharks has been worked out beyond this period by Ayers ('92), and it is very clear from its mode of growth that it is directly related to the canal organs of the lateral line.

A consideration of the so-called branchial sense-organs and their ganglia is reserved to be published later with the part on the Nerves.

NOTE. — I have been indebted to the persons mentioned below for material that has helped fill up the gaps in my collection of embryos, and I desire here to express my appreciation of their kindness.

To Miss Julia B. Platt, for the loan of sections of Acanthias; to Dr. E. L. Mark, for young stages of Squalus; to Mr. Frank Smith, for a considerable number of embryos; to Professor J. E. Reighard for the loan and free use of his entire collection of elasmobranch embryos; to Professor A. D. Morrill, for early stages of Squalus.

LAKE FOREST, ILLINOIS,
January, 1895.

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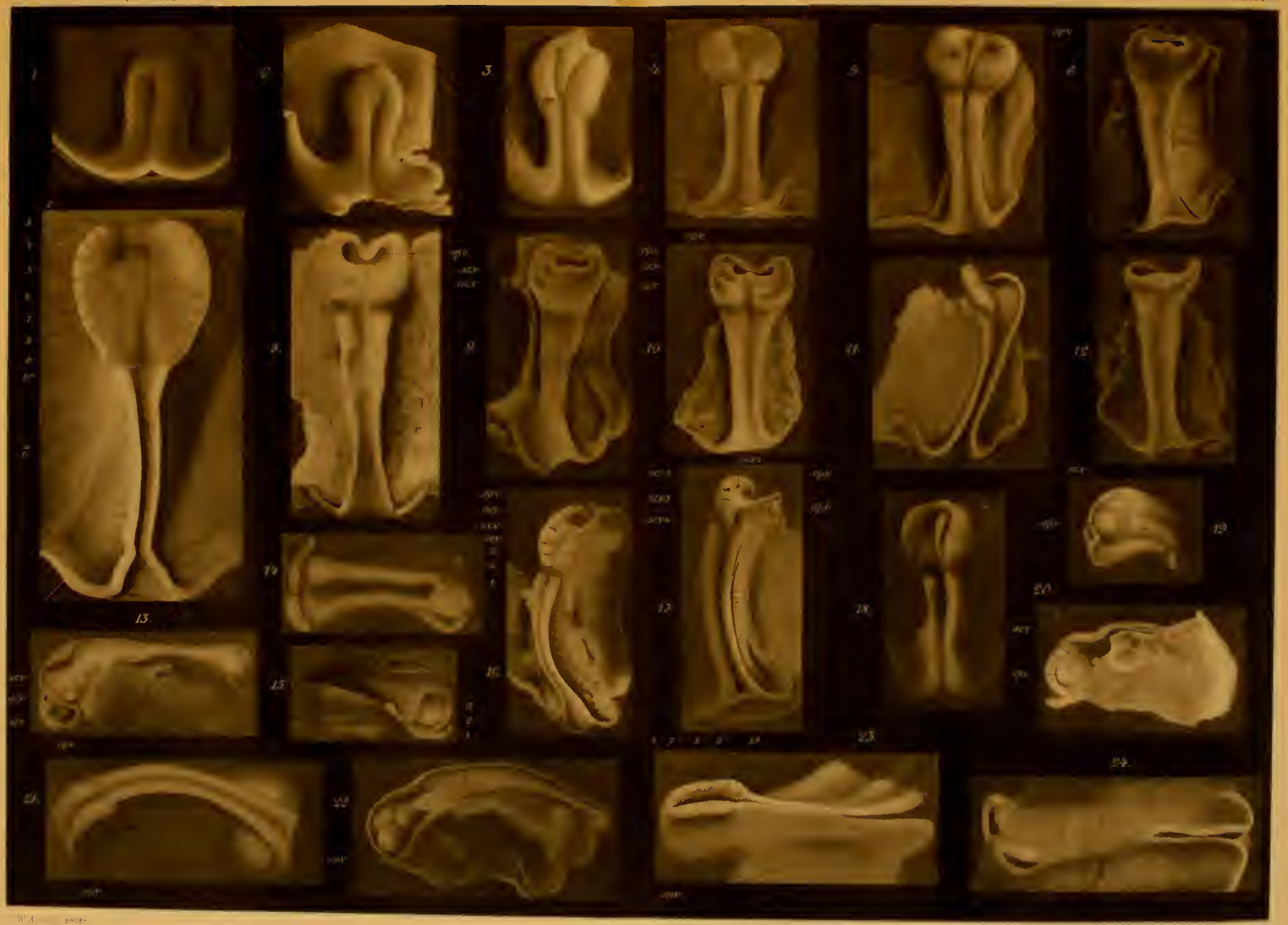
EXPLANATION OF FIGURES.

Reference Marks.

1, 2, 3, 4, etc. Metameric segment.
AA' line drawn in front of the anterior neuromere of the vagus nerve.
au. auditory vesicle.
ac. v. accessory optic vesicle.
al. c. anterior portion of alimentary canal.
cbl. cerebellum.
cbr. prosencephalon.
ep. epiphysis.
H. C. mandibular head cavity.
mb. mid-brain.
mes. mesoblast.
met. metameric segment.
na. nasal epithelium.
op. n. optic nerve.
op. s. optic stalk.
op. v. optic vesicle.
p. paraphysis.
so. serial sense-organ.
Thl. thalamencephalon.
Thl. op. thalamus opticus.
ven. 4. fourth ventricle.
Zp. Zirbelpolster.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EXPLANATION OF PLATE XXVI.

The figures are from untouched negatives and are noteworthy in showing the very early condition of the optic vesicle, the accessory vesicles and in some cases the primitive metameric segments. They are all photographs of *Squalus acanthias* and, with the exception of Fig. 7, are × about 20 diameters.

FIG. 1. Photograph of embryo between Balfour's stages *B* and *C*.

FIG. 2. Somewhat older embryo. The embryonic rim on the left side shows faintly some of the metameric segments (not reproduced by the artist).

FIG. 3. Slightly older embryo to show the formation of the head-plate and the central wedge-shaped process thereon. This is the stage in which the neural folds are started along the margins of the body. They are ventrally curved.

FIG. 4. Stage with a rounded head-plate when the optic vesicles first become evident.

FIG. 5. Another embryo, about the same age as the preceding, in which the optic vesicles and one of the accessory vesicles are shown.

FIG. 6. Somewhat older embryo showing the infolding for the optic vesicles extending across the median plane.

FIG. 7. Older embryo somewhat higher magnified. The head-plate is very broad, the trunk narrow. The neural folds of the head lie nearly in the horizontal plane. The metameric segments show well on the left margin of the head-plate. They exist in the earlier stages, but are very difficult to catch with the camera.

FIG. 8. Specimen in which the depressions for the optic vesicles show very distinctly.

FIGS. 9 and 10. Two embryos older (although smaller) than the preceding. In both two pairs of accessory optic vesicles are to be seen on the cephalic plate back of the primary optic vesicles.

FIGS. 11 and 12. Two specimens slightly older than the preceding two, seen from different point of observation.

FIG. 13. Embryo after the neural folds have begun to grow upwards, seen obliquely from the left side. Gives an external view of the vesicles on one side and an internal view of them on the opposite side of the neural folds.

FIG. 14. Embryo from which Fig. 29, Plate XXVII, is drawn. Shows metameric segments on the exposed ventral surface of the neural folds.

FIG. 15. Embryo of same age as Fig. 13 and Fig. 31, Plate XXVII. Shows metameric segments on the neural folds in front of the eye vesicles.

FIG. 16. Embryo of the same age as Fig. 9 just above it. Seen in a position more favorable to bring out the accessory vesicles on the cephalic plate. The optic vesicle on the right side shows as an external protuberance.

FIG. 17. Somewhat older embryo viewed obliquely from above. Shows the optic vesicle of the right side as an external rounded protuberance, and that of the left side from within as a cup. Behind the optic vesicle on the left side of the cephalic plate are four accessory vesicles. They show their serial relation with the primary optic vesicle.

FIG. 18. Specimen showing a large development of the central tongue-like process. Embryo slightly older than that in Fig. 7.

FIGS. 19 and 20. Side view of two heads of embryos with open neural groove to show the external appearance of the optic vesicle.

FIG. 21. Embryo of same age as Fig. 13 viewed obliquely from above.

FIG. 22. Side view of embryo of the same age (with broadly open neural groove) to show the external appearance of the optic vesicle and the other vesicles behind it.

FIG. 23. Embryo after partial closure of the neural groove. Showing two openings, an anterior and a posterior one, into the neural canal.

FIG. 24. Somewhat older specimen with head broken off. Showing three openings (smaller than those in the preceding embryo) into the neural canal.


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EXPLANATION OF PLATE XXVII.

All the figures are of *Squalus acanthias;* they are drawn with the aid of the camera, and are all × 45 diameters.

FIG. 25. Young embryo intermediate between Balfour's stages *B* and *C*. The embryo so far as formed is divided into eight pairs of metameres and these are continued without break, or any change in character, into the halves of the embryonic rim. The eleventh metamere which, in later stages, lies in front of the vagus nerve is now on either side the third one from the axial embryo.

FIG. 26. Somewhat older embryo, showing the change in form of the head region. The axial embryo now includes about fifteen pairs of metameres.

FIG. 27. Slightly older than the preceding; there are about eighteen pairs of metameres in the axial part of the embryo and, as in the former instances, are continued into the embryonic rim. Two longitudinal marginal furrows have appeared, separating two marginal bands from the rest of the embryo. Along the line of these furrows are seen four depressions that mark the very beginning of segmental sense-organs.

FIG. 28. View from the upper side of the same embryo illustrated in Fig. 29. The cephalic plate is now clearly marked off from the more slender trunk region. The depressions for the optic vesicles (*op.*) have made their appearance.

FIG. 29. View of the same embryo from the ventral aspect. The yolk has been completely removed, and we get a view directly into the gastrular cavity. There are eleven pairs of metameres in the broad part of the cephalic plate. The neural folds are ventrally curved. The outlines of the figure and the neural segments are too symmetrical.

FIG. 30. Older embryo with neural folds lying in the horizontal plane. The broad cephalic plate is in marked contrast with the slender trunk.

FIG. 31. Embryo in which the neural folds have nearly attained the vertical plane. The neural groove is still open. The optic vesicle (*op.*) and the combined vesicle of mid-brain and accessory optic (*mb.* + *A. op.*) vesicle show on the sides of the head. There is also the beginning of mandibular head cavity (*H. C.*) and the branchial pouch. The original metameric divisions are still very plain.

FIG. 32. Embryo just after the closure of the neural groove in the anterior end. The posterior part of the neural canal is not completely closed. Note the metameric divisions indicated by numbers *1*, *2*, *3*, etc.

FIG. 33. Embryo after complete closure of the neural groove and before the appearance of the ear vesicle.

FIG. 34. Embryo after the differentiation of the ear saucer. The five anterior metameric divisions are no longer distinguishable, those of the hind-brain are prominent and are approximated in the middle plane. One gill-cleft has broken through. The nasal pit has started.

FIG. 35. Slightly older embryo showing several characteristic changes. The line of neural segments are being forced apart by lateral growth of the roof of the hind-brain. The fifth nerve is plainly visible. Over the gill-clefts runs a continuous nodulated thickening containing the branchial sense-organs and the rudiment of the lateral line.

FIG. 36. Somewhat older embryo differing from the preceding mainly in showing the rudiments of the seventh, eighth, ninth, and tenth nerves. Note the lens and choroid fissure in the eye vesicle.

FIG. 37. Slightly older than the preceding. The line of neural segments are undergoing some changes whereby the concavity on the lower margin is made to correspond with a crest on the upper margin. In embryos of about the age represented in Fig. 36 or a very little older the epiphysial outgrowth arises from the roof of the thalamencephalon.

EXPLANATION OF PLATE XXVIII.

A series of partial dissections of embryos of *Squalus* in such a way that the brain-walls have been laid bare. Figs. 46–49 × 45 diameters. Figs. 50–56 × 10 diameters.

FIG. 38. Embryo with open neural groove. The epidermal layer and the mesoderm have been removed from the sides of the brain-wall; behind the optic vesicle is seen a bilobed protuberance — the combined mid-brain vesicle and the anterior accessory optic vesicle.

FIG. 39. Older embryo with neural canal partly formed. The specimen shows the same condition of mid-brain vesicle and that of the anterior accessory optic vesicle.

FIG. 40. The brain-walls of an embryo of the same age as that shown in Fig. 32.

FIG. 41. Embryo before dissection, showing especially well the contours of the brain-walls.

FIG. 42. The same embryo after exposure of the brain-walls by dissection. The thalamencephalon is well exhibited. The mid-brain is bilobed.

FIG. 43. Sketch of partially dissected embryo just after the appearance of the auditory vesicle.

FIG. 44. The exposed brain-walls of an embryo slightly older than that represented in Fig. 34.

FIG. 45. Brain of embryo about same age as that in Fig. 35. The auditory vesicle has been left in position. The mid-brain is now indistinctly trilobed.

FIG. 46. Brain-walls of embryo with the optic vesicle removed. About the same age as the preceding.

FIG. 47. Dissection of the brain of the embryo of which Fig. 36 is an external view. The mid-brain is distinctly trilobed. There are eight clearly marked segments in the hind-brain.

FIG. 48. Dissection of embryo slightly older than the one represented in Fig. 37. The thalamencephalon is now definitely marked out by furrows; it bears upon its summit two rounded confluent protuberances.

FIG. 49. Dissection of brain of embryo somewhat older than the preceding. The thalamencephalon is clearly defined. The posterior protuberance from its roof has grown much faster than the anterior one. The former is the beginning of epiphysis. There are nine neural segments in the hind-brain.

The embryos are now too large to represent advantageously on the same scale, and in the following figures the scale of magnification is reduced from 45 to 10 diameters.

FIG. 50. Shows embryo of same age as that represented in Fig. 32.

FIG. 51. Embryo of nearly the same age as that represented in Fig. 34.

FIG. 52. Partly dissected embryo of about the same age as that represented in Fig. 36 and again in Fig. 47.

FIG. 53. Brain-walls of an embryo just older than that shown in Fig. 49.

FIGS. 54 and 55. Successively older embryos to show especially the changes in the thalamencephalon and the outgrowth from its roof of the epiphysis.

FIG. 56. The same brain shown in Fig. 55, with the cerebral lobes removed and turned so as to view directly against the epiphysis.


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EXPLANATION OF PLATE XXIX.

Figs. 57–62 a continuation of the series of dissections shown on the two previous plates. All × 10 diameters. Figs. 65–87 × 45 diameters.

FIG. 57. Side view of brain of an older embryo than that sketched in the foregoing figure. The neural segments have now become obliterated, and the three lobes of the mid-brain (secondary divisions) are no longer distinguishable. The epiphysis is well developed; in front of it is a semicircular fold of the brain-wall; this structure is the remnant of the elevation which started in front of the epiphysis on the roof of the thalamencephalon (see Figs. 48 and 49). It has become reduced by compression between the rapidly growing adjacent brain regions. The paraphysis is also visible in this drawing.

FIG. 58. View of the same brain from above looking into the cavity of the fourth ventricle.

FIG. 59. The same brain after removal of the cerebral lobes and arranged so as to show the epiphysis in front view.

FIG. 60. Brain of older embryo showing substantially the same features as the preceding.

FIG. 61. The cerebral lobes of the same brain after removal. Note the paraphysis arising from the posterior part of the roof of the prosencephalon.

FIG. 62. The same brain after removal of prosencephalon and arranged so as to show to best advantage the epiphysis with its stalk.

FIG. 63. Horizontal section of embryo shown in Fig. 25 to show the general appearance of the metameres in section. × 50.

FIG. 64. Horizontal section of embryo sketched in Fig. 26 showing metameres in section, and adjacent layer of mesoderm. × 50.

FIG. 65. Section of head of *Torpedo ocellata* through the optic vesicles at the time of their first appearance. Compare this with Zieglers' Pl. IV, Fig. 19[1].

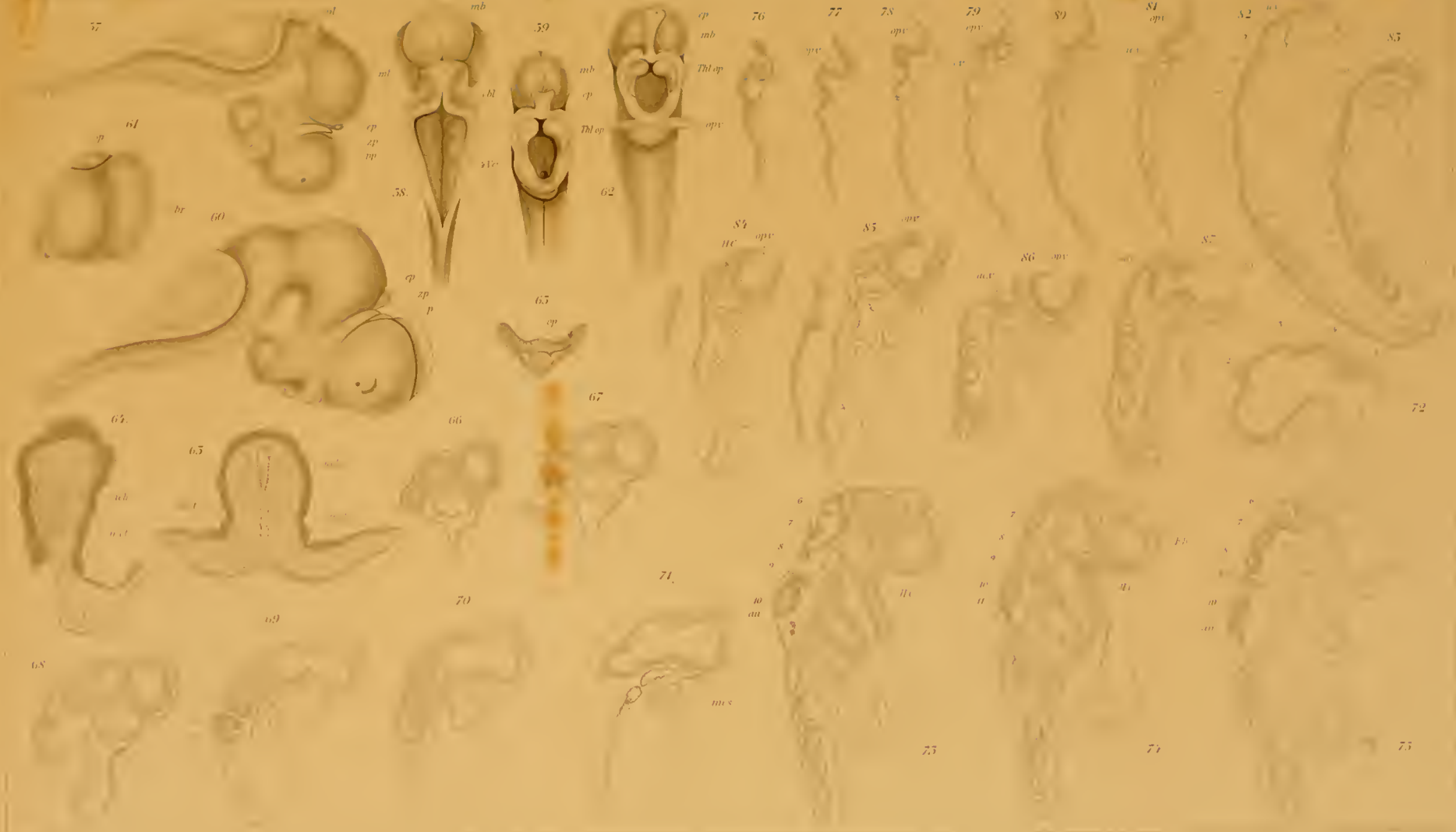
FIGS. 66, 67, 68, 69, 70. Successive sagittal sections of embryo just after closure of the neural groove and prior to the appearance of the ear vesicle. Show the primary fore-brain, the mid- and hind-brains, and three very prominent neural segments of the hind-brain. The segments are the seventh, eighth, and ninth respectively. When the ear is first differentiated it arises opposite the ninth segment.

FIG. 71. Deeper section of the same embryo showing a curved line of mesoderm over the mandibular cavity.

FIG. 72. Sagittal section of embryo near the median plane after the formation of the auditory saucer and the complete closure of the neural groove. The five neural segments of the fore- and mid-brain are exhibited. The second neural segment nearly coincides in position with the neuropore.

FIGS. 73, 74, 75. Three successive sagittal sections of an embryo of about the age of that drawn in Fig. 34. It is slightly older, shows well the neuromeres of the hind-brain. The ear capsule is in the space of the tenth neuromere. In front of it in Figs. 73 and 74 are seen the roots of the seventh and eighth nerves.

FIGS. 76–87. Sagittal sections of the specimen photographed as Fig. 17, Pl. XXVI. Giving evidence of a series of cup-like depressions on the neural plate of head and trunk. The series of these cupped areas is terminated in front by the optic vesicles. On the cephalic plate they are relatively large and resemble the primary optic vesicles in mode of origin and in structure. How far the series extends into the trunk I have not been able to determine.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EXPLANATION OF PLATE XXX.

FIGS. 88–112. Twenty-five transverse sections of an embryo very slightly older than that represented in Fig. 16. The sections 88–100 lie in the region of the cephalic plate. The following sections 105–112 lie in the neck and trunk regions.

These sections are remarkable in bringing to light serial depressions along the walls of the neural folds. They show that the serial cup-like differentiations extend back of the cephalic plate. I have not been able to determine the number of serial differentiations of this character in the embryo, but it is clear there are several pairs behind the cephalic plate upon which I have noted in surface study four pairs in addition to the eyes. × 45 diameters.

[Figs. 113–115 were drawn from nature by Miss Tanetta Gilleland. Figs. 117 and 118 are taken from Kupffer's "Studien zur vergleichenden Entwickelungsgeschichte des Kopfes der Kranioten," and Fig. 116 is after Froriep.]

FIG. 113. View of the head-end of embryo of *Amblystoma* in the open neural groove stage, × about 10 diameters. Shows segmental folds in the neural folds and a smooth neural plate.

FIG. 114. View on the caudal extremity of the same embryo.

FIG. 115. Embryo of *Rana palustris* showing large obvious segmental folds in the median plate and also smaller fainter folds in the neural ridges. The latter correspond to the segments in the neural ridges in *Amblystoma*.

FIG. 116. Embryo of *Triton cristatus* after Froriep. Showing large obvious folds in the median plate with unsegmented neural ridges. These median folds probably correspond to those in *Rana palustris* and not to the metameric divisions in the neural folds.

FIG. 117. View on the head-end of *Salamandra atra*, according to Kupffer, showing segmental folds in the median plate but none in the neural ridges. Compare with Fig. 115.

FIG. 118. Caudal end of same embryo.

Figs. 119–124 are sagittal sections of *Squalus acanthias*. × about 45 diameters.

FIG. 119. Section of head of embryo about same age as those represented in Figs. 37 and 48, showing the roof of the thalamencephalon raised into two elevations.

FIG. 120. Somewhat older embryo showing the tubular-like growth of the epiphysis.

FIG. 121. Still older stage showing reduction of the anterior part of roof of thalamencephalon and great increase in depth of the furrow in front of the thalamencephalon.

FIG. 122. Older stage in which the paraphysis has made its appearance from the roof of the prosencephalon. The choroid plexus has also started.

FIGS. 123 and 124. Two older stages showing the increase in length of the epiphysis ; its distal end is somewhat enlarged and is inserted into a concavity in the cranial roof. The paraphysis is indicated at *pp*. — The choroid plexus is considerably increased in extent and hangs into the cavity of the fore-brain.


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THE MUSCULATURE OF CHITON.

LILIAN V. SAMPSON.

Introduction.

The study of the musculature of Chiton was begun at the suggestion of Professor Lang at Zürich, and to him and to Dr. Karl Fiedler I am greatly indebted for advice and assistance during the first part of the work : the study was completed under the direction of Professor Morgan, of Bryn Mawr College, who has furnished me with material in addition to that originally procured by Professor Lang, and has greatly aided me by his kind interest and assistance. I am glad to have this opportunity to acknowledge Professor McMurrich's kindness in looking over the manuscript.

The anatomy of Chiton has been carefully worked out by Haller; he has described in detail the digestive tract, the circulatory system, the nervous system, the nephridia, the reproductive system, and also the muscles of the walls of the internal organs. The chief muscles of Chiton are those of the shell, of the foot, of the mantle, and of the radula. The muscles of the shell, foot, and mantle are attached to the shells, and it is therefore important to bear in mind the arrangement of the shells.

On the dorsal side of Chiton are seen the exposed surfaces of eight distinct shells encircled collectively by the mantle. Shells III to VII inclusive (reckoning from anterior to posterior) are plates arched over the back of the animal (Fig. 1, V); the anterior portion of shell II, which is longer antero-posteriorly than any of the other shells except the terminal ones, is curved over the anterior end of the body, while shells I and VIII (Fig. 1, I and VIII) cover the curved ends of the body to the edge of the mantle where it passes around the ends of the animal. The lower layer of each shell (except I) is continued anteriorly on either side under the shell next in front

of it, as an "apophysis," so that the shells deeply overlap at the sides like scales. (Fig. 1, *ap*). When the animal is extended, each shell covers the apophysis of the next posterior shell and also a portion of the outer layer of the same shell posterior to the apophysis. When the animal is contracted, only the apophyses are covered, and the shells, therefore, do not overlap in the middle line. The edges of the shells that are bordered by the mantle (*i.e.*, the anterior edge of the first, the posterior edge of the last shell, and the lateral edges of the intervening shells) are notched and firmly inserted in the muscular tissue, and a small ridge of the mantle folds over their extreme edges (*cf.* Figs. 4, 5, 7, 8, *etc.*). This general description applies to the various species which I have seen;[1] the principal variations in detail are in the degree of curvature of the shells over the back and ends of the animal, and in the thickness of the shell.

The muscles have been worked out chiefly in transverse and sagittal sections of C. olivaceus (found at Naples), though horizontal sections and dissection of the same species have aided in confirming the results; dissections of C. viridis, Spengler, a species from Jamaica that very closely resembles C. olivaceus, were of still greater assistance, as the animals are larger. An individual of this species is represented in Fig. 2 (the specimen was about 5 cm. in length after preservation in alcohol). All the figures of sections are taken from preparations of C. olivaceus.

After writing the description of the muscles, an opportunity occurred for making observations on living Chitons from Woods Holl, probably C. apiculatus.

The animal, never rapid in its movements, is capable of assuming the greatest variety of positions, notwithstanding its dorsal plate armor. It crawls slowly by means of the foot, and can travel through the arc of a comparatively small circle. When disturbed, it attaches itself firmly to the surface on which it crawls. If the animal is removed, it rolls itself together until the ventral surface of the anterior and posterior ends of the body

[1] C. pellis serpentis, C. olivaceus, C. granulatus, C. cajetanus, C. viridis, Spengler.

are flattened against each other, or even overlap, in which case the natural position seems to be with the posterior end curled under the anterior; if the anterior edge of the mantle folds under the posterior, the animal may several times open and close, the posterior edge getting more and more nearly under the anterior at each attempt, until finally the posterior edge is entirely folded under, and the animal remains quiet. It lies thus rolled together for some time on the bottom of the dish or on the stone; in trying to recover its footing, it is able to arch the ventral surface of the foot, thus making its dorsal side bearing the shells concave, to such an extent that the ventral surface of both anterior and posterior edges of the mantle is turned under, and rests on the stone or other surface on which the animal has been lying; this position is not retained very long at a time, but is repeatedly assumed after intervals of rest in the contracted state. Such attempts to regain the normal position do not appear to be successful unless by its exertions the animal may happen to move to a part of the stone where it can lay hold of some irregularity of the surface. The Chiton, when on its back, can twist itself into most contorted shapes, and may finally attach itself to a stone if the stone lies near by.

Muscles of the Shells.

Changes in the relative positions of the shells are brought about by muscles immediately ventral to the shells, which can be removed in somewhat imperfectly preserved specimens. The muscles are then seen exposed on the dorsal surface (Fig. 2).

They are: (1) A median dorsal longitudinal muscle (Figs. 2, 3, also 4, 5, 10, *md*), whose fibres pass for the most part from the anterior edge of each shell, between the apophyses, to the ventral surface of the anterior part of the next anterior shell, though some fibres are continuous under the junctions of the shells; this muscle is thickest on either side of the middle line, except at the point of the posterior attachment (*cf.* Fig. 4, *md* with Fig. 5, *md*).

(2) A pair of oblique dorsal muscles (Figs. 2, 3, 4, 5, 6, *od*) attached with the median dorsal muscle to the anterior edges of shells VIII–II, inclusive. These muscles run obliquely forward, at first along the edges of the apophyses of the shell to whose median anterior edge they are attached, and anteriorly they are attached to the next anterior shell (under which they have taken their course) on the line where its apophyses begin (Figs. 2, 3, *od*); *e.g.*, one of these muscles is attached to the anterior edge of V, runs obliquely forward under IV, and is attached to the ventral surface of IV where its apophyses begin. Both the median and oblique dorsal muscles are wanting under VIII, as no shell posterior to it occurs. Under II, the median dorsal muscle is comparatively narrow and thick (Fig. 10, *md*), for it is crowded toward the median line by a group of muscles (*dr*) extending from II to the radula; under the more anterior part of the shell, the fibres diminish in number, so that the anterior limit of the muscle is at the beginning of the curvature of the shell over the end of the body (*cf.* Fig. 3 and description of the shells). The anterior attachment to the shell of each of the oblique dorsal muscles under II is not continuous, but the fibres are attached in groups that alternate with the attachments of groups of fibres of pedal muscles (v. *infra*, see Fig. 3): this is also true to some extent of the corresponding muscles under the other shells. Under shell I are two pairs of muscles that pass anteriorly (and also ventrally, following the shape of the shell) from the anterior edge of II; the muscles of the median pair diverge but slightly from their attachment to II, and represent the median dorsal muscle (Fig. 3, *md*); the more lateral pair (Fig. 3, *od*) corresponds to the oblique dorsal muscles of the other shells, but each muscle of the pair is divided very near to its attachment to II into two main strands; the outer fibres of the outside strands end at the shell with alternating groups of fibres of a dorso-ventral muscle (*cf.* Fig. 3).

(3) At the lateral edges of the shells, longitudinal muscles (Figs. 2, 3, *ll*, and also *ll* of cross-sections) on either side which pass from the ventral surface of one shell to the dorsal surface of the apophysis of the next posterior shell. As in the

case of the median dorsal muscle, some fibres are continuous under the overlapping edges of the shells.

(4) Between the shells where they overlap, a thin cushion of muscles covering dorsally the apophysis, and having therefore the same outline. The arrangement of muscles forming this cushion is complicated: at the antero-lateral edge of the apophysis are oblique fibres (Figs. 4, 6, c_1) lying in the transverse plane, which pass from the ventral shell (apophysis) obliquely toward the median line, to be attached to the dorsal shell. Further relations of these fibres to a large muscle in the mantle will be described in connection with the muscles of that organ. The fibres of this border of muscles at the edge of the apophysis are gradually replaced by fibres in the sagittal plane (Figs. 4, 6, c_2), which are attached anteriorly to the ventral shell and pass to a more posterior attachment to the dorsal shell. Between the ventral shell and the posterior ends of these fibres is a region occupied by a set of fibres in the horizontal plane (Figs. 4, 6, c_3) that are also attached by their anterior ends to the ventral shell and pass laterally and posteriorly to be attached by their posterior ends to the dorsal shell.

I have endeavored by comparing measurements of several specimens killed in a contracted state with those of animals killed while extended, to determine the functions of the various shell muscles, but have been unable to obtain any satisfactory results because of the small size of the animals and the variations in the proportions of different individuals. The following account is a suggestion of the possible mechanism of the shells, based on observations on the positions of the muscles. When the animal is contracted or extended, the relative lengths of the lines on the parts covered by the shells must be very much like those of the finger when bent or straight: the median dorsal line is longest when the finger is bent, and at the same time the median ventral line is shortest, while some line between the two remains constant in length, whatever the position of the finger, *i.e.*, the line, probably, along which there are the fewest wrinkles of the skin. If the finger were covered by strips of paper shaped like the shells of

Chiton, and projecting at the sides under one another, we should have a rude representation of the condition of the shells. The median dorsal muscle I regard as probably an extensor, for the median dorsal line is obviously increased in length when the animal is contracted, and conversely diminished when the animal is extended. The muscle is attached from anterior to anterior edge of the shells, so that it is impossible that the muscle by its contraction should draw two shells at an angle to each other and itself form the third side of the triangle, as might occur if the muscle went from the anterior edge of one shell to the posterior edge of the next posterior shell (from the tip of the finger to the second joint). It seems probable that the oblique dorsal muscles likewise function as extensors. The lateral longitudinal muscles, on the other hand, are on a line that is diminished when the animal is contracted, for the maximum overlapping of the lateral edges of the shells occurs at that time; these muscles, then, are to be regarded as contractors.

It is reasonable to suppose that these last two muscles, and also the muscles of the cushion, are used in motions that involve the two sides of the animal in different ways, and it is possible that this is the chief function of the oblique dorsal.

The muscles of the cushion in the sagittal and horizontal planes (c_2 and c_3) probably serve to readjust the shells at the extension of the animal. The median dorsal tends to draw the anterior shell over the posterior, and does not control the posterior edge of the shell. The sagittal muscles (c_2) of the cushion, acting on either side of the median line, and the horizontal muscles (c_3) controlling the shells more laterally where they are curved, tend, by virtue of their oblique position, to draw the posterior shell from under the anterior, and at the same time to draw the surfaces of the shells together; or, in other words, these muscles lie in the direction of the resultant of a force parallel with the surfaces of the shells, and of a force perpendicular to the shells. The muscles in the transverse plane at the antero-lateral edge of the cushion, perhaps serve to draw the antero-lateral edge of the apophysis nearer to the dorsal shell on contraction of the animal, a motion

opposed to the action of the sagittal and horizontal cushion muscles (which draw the posterior part of the dorsal shell nearer to the ventral) in the extension of the animal.

Muscles of the Foot.

The muscles to the foot are attached to the ventral surface of the shells, on either side, between the body cavity and the mantle, whence they pass in various directions into the foot and are dispersed through the whole organ; some of the fibres cross the middle line, and the body cavity is bounded by muscle fibres below and at the sides (Figs. 4, 5).

Corresponding with the regularity in the relations of the shells, the muscles that occur under one shell are exactly repeated under each of the others, except where the regularity is interrupted by other organs. For any cross-section of shell IV, with the underlying muscles, there is an exactly corresponding section of shells V and VI. The same order of muscles is easily recognized under III and also under VII, where, however, the connections between the auricles and the branchial veins, and the efferent ducts of the nephridia and reproductive organs introduce modifications; I and VIII are different, owing to their terminal positions, and the buccal muscles under II (and also under I) disguise to some extent the usual order, although, even in these cases, muscles corresponding to those under the other shells occur.

Shell VI.

I shall first describe the muscles under a typical shell — for example under VI — and shall limit the description to the muscles of one side of the animal (the bilateral symmetry being perfect).

The muscles are divided into two groups, which, near the shell, are separated from each other (*cf.* under V, Figs. 6, 2, right side). Owing, however, to the diverse directions in which the fibres run, the muscles form at a lower level a compact mass from end to end of the animal, completely occupying the foot and the narrow space between the mantle chamber and body cavity.

The anterior group of muscles is attached to the shell in the region where it emerges from under the shell next anterior to it (Fig. 3, *aga ; cf.* also Figs. 2, 6); the posterior group to the region anterior to the underlying edge of the apophysis of the next posterior shell (Fig. 3, *pga ;* Figs. 2, 6). Nephridial branches occupy, as a rule, the spaces immediately ventral to the shell and alternate with the groups of muscular attachments.

In each group, at the shell, are three muscles that correspond in the two groups, although the exact relations of the muscles to one another are not identical. The muscle nearest the median line at the shell will be called (because of its distribution) the "latero-pedal," the most lateral muscle, the "medio-pedal," and the muscle between, the "antero-oblique."

The inner fibres of the latero-pedal (Figs. 4, lp_1, 5, lp_2) are attached to the shell over the body cavity (so that they form the roof of the lateral part of the cavity), curve out, and then pass ventrally into the foot ; the remaining fibres of the muscle are, on the whole, dorso-ventral. After leaving the shell, some of the fibres diverge anteriorly and posteriorly (Figs. 2, 6, lp_1, lp_2) until those of consecutive anterior and posterior groups meet ; the muscle is distributed to that part of the foot which lies outside the pedal nerve, including the portion spread out under the mantle chamber (Figs. 4, 5). The posterior fibres of the latero-pedal of the anterior group, and the most lateral fibres of the oblique dorsal muscle, overlap near the shell. (Fig. 4 is taken from a section anterior to this region ; but *cf.* Fig. 3, *od* and *aga*.)

The medio-pedal muscles (Figs. 4, mp_1, 5, mp_2) are attached to the shell over the mantle chamber, and pass obliquely inward until they cross the path of the latero-pedal fibres (Fig. 6, mp_1, mp_2), and continue the boundary of the body cavity ; some fibres are distributed to the part of the foot inside the pedal nerve, and others cross the median line, and end in the opposite half of the foot. The fibres of the medio-pedal of the anterior group do not diverge anteriorly and posteriorly from their attachments to the shell like those of the latero-pedal muscles, but the muscle has a broader attachment antero-

posteriorly than the latero-pedal of the same group, extending beyond it anteriorly, and for some distance posteriorly (Fig. 13, III–VI, lp_1, mp_1 ; *cf.* also Fig. 2, lp_1, mp_1 of IV).

The fibres of the medio-pedal of the posterior group, like the latero-pedal fibres, diverge from the shell, and where these two muscles and the antero-oblique occur side by side, it is difficult to draw sharp lines of division between them, as may be seen in cross-section (Fig. 5, lp_2, mp_2). The medio-pedal muscle, however, as a whole, is relatively more posterior than the latero-pedal (Fig. 13, III–VI, lp_2, mp_2 ; also Fig. 2, lp_2, mp_2 of IV).

In the floor of the body cavity are occasional fibres that stretch horizontally across, and disappear among the fibres of the medio-pedal muscle on either side (Fig. 5).

The antero-oblique of the anterior group (Figs. 2, 4, 6, ao_1) runs obliquely forward from its attachment between the latero- and medio-pedal muscles, and the anterior and posterior limits of its attachment to the shell are relatively a little posterior to those of the latero-pedal (Fig. 13, III–VI, ao_1, lp_1). The attachments of the antero-oblique fibres of the posterior group begin anteriorly outside of the attachment of the latero-pedal muscle (Fig. 13, III–VI, ao_2, lp_2), and more posteriorly are mingled with the fibres of the latero- and medio-pedal muscles (Fig. 5, ao_2) ; the fibres run very obliquely forward, overtake those of the antero-oblique muscle of the anterior group (Fig. 4, ao_1, ao'_2 ; *cf.* also Fig. 2, ao_1, ao_2 of V), and pass ventral to them into the foot.

In the anterior group, still another muscle, the "postero-oblique" (Figs. 2, 4, 6, *po*), is attached to the shell immediately inside the attachment of the medio-pedal muscle, although it does not extend posteriorly so far as the medio-pedal and its attachment reaches relatively more anteriorly (Fig. 13, III–VI, *po*); this muscle runs obliquely backward inside the medio-pedal of this group (Fig. 4, *po*), but outside the medio-pedal of the posterior group (Fig. 5, po').

There are, then, two groups of muscles to the foot under the typical shell, each composed of three muscles having a similar distribution in the two groups, but differing in the

relative anterior and posterior limits of their attachments ; in the anterior group a fourth muscle occurs not represented in the posterior group. Fibres in the thick part of the foot that in cross-section appear to be the cut ends of longitudinal muscles, are in reality the continued fibres of the oblique muscles, and arise in part, also, from a horizontal muscle from VIII and an oblique muscle from I and II that extend a long distance into the foot. Both will be described among the muscles of the shells to which they belong. Longitudinal fibres which are independent of all these muscles occur in the most ventral part of the foot and in its lateral edge ; and, in the network of muscles in the ventral part of the foot, are additional diagonal and horizontal fibres in the transverse plane (see cross-sections). This general description applies to the three shells, IV, V, and VI.

Shell III.

The arrangement under shell III is scarcely modified. The antero-oblique muscles, in this more anterior part of the body, are reduced in extent, and the fibres are perhaps more perpendicular in direction ; on this account the antero-oblique fibres of the posterior group are in cross-section less distinguishable than in the typical case from those of the latero- and medio-pedal muscles. The postero-oblique muscle, on the other hand, is large and conspicuous ; the corresponding muscle of II is also large, and still prominent at its lower level under III.

Shell VII.

Under VII the genital duct crosses to its external opening into the mantle chamber beyond the gill, anterior to the attachment of the medio-pedal of the anterior group, but ventral to the anterior part of the attachment of the postero-oblique. Thus the attachment of the medio-pedal is interrupted, and the medio-pedal does not extend anteriorly beyond the latero-pedal (Fig. 13, VII). Posterior to the anterior group of muscles the auricle extends to the branchial vein and the nephridial duct to its external opening into the mantle cham-

ber, and the attachments of the muscles are accordingly equally limited posteriorly ; the branchial artery sends a branch toward the body cavity in the same region (Fig. 13, VII, attachments of the anterior group).

The nephridial duct and extended auricle cut off also the anterior portion of the attachment of the latero-pedal and antero-oblique muscles of the posterior group, until the anterior limits of these attachments are relatively posterior to the anterior limit of the medio-pedal (Fig. 13, VII, posterior group). The postero- and two antero-oblique muscles thread their way between the various ducts and blood vessels as strands rather than bands of muscles, as under the other shells.

Thus under VII not only is the space between the muscular attachments occupied by organs other than the nephridial branches, but the attachments even of the muscles to the shells are reduced, and the oblique muscles are compressed into small spaces between the ducts and vessels ; notwithstanding these modifications, however, muscles corresponding to all of those under the typical shell can be clearly recognized, and have a perfectly typical distribution.

Shell VIII.

Under VIII the postero-oblique muscle is merely a small group of fibres, not entirely wanting, as I at first supposed. The antero-oblique fibres, on the other hand, are especially prominent. There is no sharp division of the muscles into two groups, but the latero- and medio-pedal fibres form almost continuous muscles from the anterior to the posterior limits of their attachments : both muscles begin on the ventral surface of the most anterior portion of the apophysis, a point which is relatively in front of the usual attachment even of the medio-pedal, under the other shells[1] (Fig. 13, VIII). Toward the posterior part of shell VIII the muscles of the opposite sides gradually approach (corresponding to the narrowing of the

[1] In a preceding account, a sufficiently anterior limit was not attributed to these muscles. It should also be noted that in the diagram, Fig. 2 of the account referred to, the median dorsal muscle of shell II is extended too far anteriorly (*Jenaische Zeitsch. f. Naturwiss.* xxviii, Bd. N.F. xxi).

shell) and are limited posteriorly by a muscle of the mantle around the end of the body. An anterior antero-oblique muscle is well defined. At the level of the narrowest part of the foot, within the latero-pedal muscle and hence bounding the body cavity, is a powerful horizontal longitudinal muscle that is attached to the shell (Fig. 13, VIII, *hl*) as it forms the dorsal curvature of the end of the body, and extends anteriorly into the foot : its attachment to the shell reaches the median line, and thus the muscle meets its fellow of the opposite side of the body, over the union of the branchio-visceral nerves. At a higher level than the horizontal muscle, fibres are attached to the shell among and outside the fibres of the latero-pedal muscle (Fig. 13, VIII, *ao*) and pass anteriorly and slightly obliquely into the foot, taking the place of the antero-oblique muscle in the posterior part of the shell.

Shell II.

Under II a condition the reverse of that under VIII, at the opposite end of the body, is found among the oblique muscles, since the postero-oblique is very prominent and antero-oblique fibres are wanting ; the antero-oblique were seen to have diminished already under III, where the muscle of that direction in the anterior group is so reduced as to be no longer recognizable as a distinct muscle when the fibres have passed anteriorly under II.

The attachments of the fibres of the oblique dorsal shell muscle are in groups among the latero-pedal fibres of the anterior group, and some of the oblique dorsal fibres reach so far laterally as to interrupt the posterior part of the attachment of the postero-oblique (*cf.* Fig. 3 and Fig. 13, II).

Some of the attachments of the muscles of the anterior group are interrupted also by muscles to the buccal mass, and the relations of their anterior limits altered : a large radula muscle (perhaps the " sphincter oris " of Haller), attached far out in the mantle to II (Figs. 10, 9, *or*, 3, *ora*), passes obliquely forward to the buccal mass (" oblique radula muscle "). Immediately ventral to this muscle where it has reached the

region of the attachment of the latero-pedal, another buccal muscle ("lateral protractor") passes across from the mantle to the buccal mass (Fig. 3, *lpra*, Figs. 9, 11, *lpr*). The anterior limit of the attachment to the shell of the medio-pedal fibres is relatively posterior to that of the latero-pedal fibres (Fig. 13, II), for the oblique radula muscle, approaching the anterior group from the lateral edge of the shell, cuts off the attachments of the outer muscles of the group obliquely, and, with the lateral protractor muscles, passes anterior to the latero-pedal fibres to reach the buccal mass (Fig. 9, *or*). Under the posterior part of II (or most anterior edge of the apophysis of III), medio-pedal fibres that bound the body cavity are interrupted, on a line with the narrowest part of the foot, by a thick muscle to the buccal mass ("posterior lateral" Figs. 10, 12, *pl*).

In front of the region of the anterior group, the buccal musculature occupies the large space over the posteriorly slanting fibres (Fig. 9, *o*) of an oblique muscle from I and II. The anterior group of fibres (here including the latero- and medio-pedal with the postero-oblique, but not the usual antero-oblique) occupies under II a relatively posterior position (*cf.* Fig. 3), *i.e.*, it lies much nearer to the posterior group, and farther from the anterior edge of the apophysis than under the other shells. The foot proper ends, and the "head-fold" takes its place in front of the region of the anterior group, so that this group is in a position to supply the foot itself.

The head-fold is joined to the body of the animal in the same way as the foot, spreading out under the mantle chamber ; anteriorly it is parallel with the edge of the mantle, and the portion spread under the mantle chamber is continued posteriorly as a lobe on either side, dorsal to the foot ; the projecting edge of the foot is rounded anteriorly, ventral to the head-fold (*cf.* Fig. 9, where the head-fold and the underlying anterior edge of the foot are met in cross-section), and in the center of the head-fold lies the mouth, bordered by a thick circular lip. The muscles, therefore, under the anterior half of II and under I have to do with the head-fold and lips, and not with the foot proper ; one exception to this, however, is to be noticed.

Shells I and II.

Corresponding in distribution with the horizontal longitudinal muscle (attached to shell VIII) of the posterior end of the body is an oblique muscle from I (Fig. 7, o_1), attached immediately anterior to the edge of the apophysis of II; this muscle and another dorsal to it, attached to the most anterior part of the apophysis of II (perhaps to be regarded as another section of the same muscle), pass together obliquely into the foot itself. Although oblique in direction, the muscle does not correspond with the postero-oblique muscles of the other shells, but, like the horizontal muscle under VIII, is different from anything to be found elsewhere in the animal; the muscles appear to have corresponding functions at the opposite ends of the body. The oblique muscle under I is attached outside the attachments of certain "dorso-ventral" fibres that appear to correspond to latero-pedal fibres of the other shells; to this extent, it resembles postero-oblique muscles, but unlike them, it passes into the foot between and not outside of the groups of "dorso-ventral" fibres (Fig. 7, o_1 and *dv*). Furthermore, the oblique muscle is attached to so posterior a part of the shell, that it must belong to a posterior rather than to an anterior group of muscles, and it will be remembered that a postero-oblique muscle is found under the other shells with the anterior group only. Finally, if, as seems probable, this muscle is completed by the oblique muscle attached to II, it differs from all the other muscles of the body, in being attached partly under one shell, partly under another. An oblique muscle passing backwards in the posterior group under the other shells would interfere with the apophysis in the movements of the shells, while the relative positions of the first and second shells appear to be less variable than those of the others; under all the shells, except I, a space occurs anterior to the edge of the apophysis.

Shell I.

To understand the distribution of the muscles under I, it is necessary to bear in mind that ventral to it the radula, with its enormous supply of muscles, occupies the cavity of the head region ; that large bundles of buccal muscles pass from the shell ; that the mouth and lips occupy a portion of the ventral surface ; and that the œsophageal ring (giving off the branchio-visceral and pedal branches) occurs in this region ; also, that the head cavity gradually diminishes in width, toward its anterior end, and that the line on which the muscles are attached is not straight, but curved, *i.e.*, parallel to the anterior edge of the mantle or shell (*cf.* Fig. 3). To the posterior part of I is attached a "dorso-ventral" muscle immediately anterior to II ; portions of its fibres pass either side of the œsophageal ring and of the oblique muscle to the foot (Fig. 7, *dv*), and are distributed to the lateral portions of the head-fold and in part to the lips, corresponding to the latero-pedal fibres of the posterior group of the other shells. Slightly anterior to these, is another group of "dorso-ventral" fibres, that passes entirely outside the œsophageal ring: these fibres may be referred to the latero-pedal fibres of the anterior group, although no sharp division exists between them and the more posterior fibres. The more anterior dorso-ventral fibres are attached to the shell with fibres of the oblique dorsal muscle (*cf.* Fig. 3), just as the latero-pedal of the anterior group under the other shells is connected with the anterior attachment of the most lateral oblique dorsal fibres. I have called these groups of fibres the dorso-ventral muscles, as a matter of convenience, although their direction is rather oblique than dorso-ventral ; the muscles in the region of the buccal mass are crowded to a relatively more lateral attachment to the shell than is the case with the corresponding muscles under other shells. Fibres that can be roughly compared to the medio-pedal muscles ("horizontal fibres," Fig. 7, *h*) are attached to the lateral portion of the shell, far out in the mantle, the fibres lying almost in the horizontal plane ; this position is possible in the region of the body in which the fibres occur, for the gills, with their nerve

and blood supply, are absent, and the mantle chamber is low. There is no division of fibres of this description into anterior and posterior groups, but the fibres are found more or less interruptedly under a large part of shell I, and posteriorly even under the anterior part of shell II (Fig. 9, *h*).

More anteriorly, where the shell becomes considerably narrower, are two additional sets of dorso-ventral muscles (Fig. 3, *dv'a*, *dv''a*, which, with the dorso-ventral muscles already described, may be regarded as parts of one system, making a condition similar to that under VIII; under I, the system is divided into three parts by two large groups of horizontal muscles to the radula, that are attached to the anterior part of the shell and cross between the groups of dorso-ventral fibres to the buccal mass (*cf.* Fig. 3 and Fig. 13, I). The most anterior dorso-ventral muscle (*cf.* Fig. 3, *dv'a*) is separated from the corresponding muscle of the other side, by an oblique median muscle to the anterior lip (Figs. 3, *omla*, 8, *oml*); and a small horizontal muscle (Fig. 8, *hml*) attached more anteriorly to I, runs in the median line, also to the anterior lip.

The lips are furnished with other conspicuous muscles. (1) Circular muscles (Figs. 7, 8, *cl*), most of which form an incomplete ring around the mouth. Anteriorly where the ring is not closed the fibres pass on either side of the oblique median lip muscle and are there lost; some of the circular muscles, however, cross posterior to the mouth and pass horizontally into the foot, and a few circular fibres are found in the anterior lip.

(2) Longitudinally directed fibres (Fig. 8, *rp*) that originate in the foot and spread like fingers in the posterior lip between the circular fibres.

(3) Shorter and less prominent, spreading fibres in the anterior lip (Fig. 8, *ra*).

Muscles of the Mantle.

The mantle is armed on the dorsal surface with calcareous spicules; ventrally it is smooth and forms the boundary of the mantle chamber, in which lie the gills, and which receives the

external openings of the reproductive and excretory organs; it is protective and is used also as a water course in respiration and during the discharge of eggs and spermatozoa, as described by Metcalf.[1] "During the ordinary respiration of the Chitons, at one anterior point, and at a posterior point on the opposite side, a small tube is formed by the arching up of the mantle edge, the bottom of the tube being formed by whatever surface the mollusc is resting upon. A constant stream of water passes into the anterior tube, through the mantle chamber and out of the posterior tube. During the discharge of the sexual products, instead of one there are two posterior tubes, one on each side, in the region of the orifice of the oviduct or of the vas deferens as the case may be. The eggs, or spermatozoa, are carried out of the mantle chamber through these tubes by the ordinary respiratory current. At other times during ovulation, the whole posterior part of the mantle of the female would be raised from the floor of the aquarium and the eggs allowed to pass out freely through the wide space thus formed."

The most prominent muscle, the "interior mantle muscle" (Figs. 4, 5, 7, *etc.*, *im*), passes from the ventral surface of the shells where the mantle joins the body, and occupies the part of the mantle that borders immediately upon the mantle chamber; the muscle is found in every part of the mantle, around the entire body. To the median shells it is attached almost continuously along a line over the highest part of the mantle chamber (Fig. 3, *ima*, Fig. 4, *im*), and immediately anterior to the apophysis of each shell, the fibres reach farther in under the shell than in any other regions, so that they meet and even cross the most lateral of the medio-pedal fibres of the posterior group (*cf.* Fig. 3). As the apophysis broadens posteriorly, the fibres become continuous with those at the antero-lateral edge of the cushion of muscles dorsal to the apophysis (Fig. 10, *im* and c_1). Still more posteriorly the fibres assume their attachment to the ventral surface of the apophysis, the dorsal shell being no longer accessible because of the greater

[1] Contributions to the Embryology of Chiton. Studies from the Biological Laboratory of Johns Hopkins University, vol. V, No. 4.

width of the apophysis. For a short interval under the new shell (apophysis) the attachment of the fibres is interrupted, and in the space, blood passes to the mantle (Fig. 3). Fig. 4 shows a section immediately posterior to this interruption, where all the fibres have not resumed their attachment. The attachment is continued posteriorly along the region of the attachment of the anterior group of pedal muscles (*cf.* Fig. 3), and posterior to this a second interruption occurs where a well-defined branch of the branchial vein, and also nerves, pass to the mantle: these spaces interrupt only the attachments of the fibres, while ventral to the spaces there is no break in the continuity of the muscle. The attachment of the interior mantle muscle to the shell is continuous around the anterior edge of I (Fig. 3, *ima*, Fig. 8, *im*) and likewise around the posterior edge of VIII. Under II, the attachment is interrupted by the lateral protractor of the buccal mass (Fig. 3, *lpra*), while the oblique radula muscle passes through the space that regularly occurs for the blood and nerve supply. Under I two pairs of groups of horizontal muscles pass from the anterior part of the shell through the interior mantle muscle to the buccal cartilages.

The mantle is further supplied by bundles of fibres that radiate from the extreme edge of the shell into the fold that covers its insertion and into the interior part of the mantle (Figs. 4, 5, *etc.*), by fibres that occur in the directions parallel to the lateral (or dorsal) and to the ventral surfaces of the mantle, and by a network of fibres in the ventral portions of the mantle. Muscle fibres occur between the branchial vein and artery, and the branchio-visceral nerve, and in the lamella of the gills.

Summary.

Under IV, V, VI, are: (1) Muscles of the shell: — a median dorsal and a pair of oblique dorsal muscles, attached to the anterior part of each shell and extending forward under the next anterior shell to be attached to it anteriorly; a series of longitudinal muscles connecting the ventral and dorsal surfaces of consecutive shells at their sides; a muscular cushion

between each shell and the apophysis of the shell next posterior, composed of oblique fibres in the sagittal plane, and, under the posterior part of these, oblique fibres in the horizontal plane, both directed so that the more anterior attachment is that to the ventral shell; and also oblique fibres in the transverse plane at the antero-lateral edge of the apophysis, continuous with the fibres of a large mantle muscle where the apophysis is of sufficient width to reach the mantle. (2) Two groups of muscles to the foot, separated from each other near the shell by a space occupied by nephridial branches; each group consists of an inner latero-pedal, a middle antero-oblique, and an outer medio-pedal muscle that crosses the latero-pedal, and continues ventrally the boundary of the body cavity (each with a distribution denoted by its name); a postero-oblique muscle occurs in the anterior group, and fibres in the ventral part of the foot, not united into defined muscles, complete the supply to the foot. (3) In the mantle, a muscle from the part of the ventral surface of the shells that is immediately dorsal to the mantle chamber, interrupted at the shell for a short distance anterior to the region of the anterior group of pedal muscles, and interrupted again more extensively anterior to the region of the posterior group; fibres from the extreme edges of the shell to the mantle, and scattered fibres of various directions in the ventral part of the mantle. The deviations from the typical arrangement under the other shells will be noted.

Shell III.

The antero-oblique muscles are reduced and the postero-oblique is enlarged.

Shell VII.

Under VII the arrangement of the muscles is modified by the efferent ducts of the internal organs, and by the blood vessels that here occur. The attachment of the medio-pedal of the anterior group is limited anteriorly and posteriorly, that of the postero-oblique is limited posteriorly and the attachment of the antero-oblique is slightly cut off posteriorly: the ante-

rior portion of the attachment of the antero-oblique and of the latero-pedal of the posterior group is interrupted.

Shell VIII.

The peculiar shape of VIII and its terminal position necessarily lead to differences in the muscular arrangement; the median and oblique dorsal muscles do not occur, there is no underlying apophysis and, therefore, no cushion of muscles ventral to VIII, and no lateral longitudinal muscle extending posteriorly; there is no division into anterior and posterior groups of the latero- and medio-pedal muscles; the antero-oblique muscles are very large, the postero-oblique almost wanting. An additional horizontal longitudinal muscle either side of the median line at the level of the narrowest part of the foot is present, and above this level, among the latero-pedal fibres and outside of them, are attached to the shell oblique fibres (representing the posterior antero-oblique muscle). The usual mantle muscles occur in every part of the mantle around this shell.

Shell II.

The region under I and part of II is anterior to the foot proper, and on the ventral surface, in the place of the foot, is the head-fold (with the mouth and circular lip).

Under II, the median dorsal muscle is crowded into a small space in the median line, and its fibres do not reach the anterior portion of the shell; the fibres of the oblique dorsal muscles are broken up into groups at their anterior ends. The anterior group of pedal muscles is posterior to its usual position, and in this group, the anterior portion of the attachment of the medio-pedal and of the postero-oblique are cut off by the oblique radula muscle, joined by the lateral protractor. Antero-oblique muscles are not found anterior to III. The interior mantle muscle is interrupted for a short distance by the lateral protractor.

Shell I.

Under I the median dorsal muscle is separated into two diverging parts, each oblique dorsal likewise into two. The lateral longitudinal passes from I posteriorly to II, but is not extended anteriorly from I, because of the terminal position of the shell; an oblique muscle from the posterior part of the shell passes with a parallel oblique muscle from II into the foot. A system of dorso-ventral fibres in the head-fold is separated by horizontal buccal muscles into two small anterior groups, and a posterior group in which can be recognized representatives of the latero-pedal muscles of the anterior and posterior groups of other shells. The muscles of the two sides gradually approach as the anterior end of the body becomes narrower; fibres corresponding to the medio-pedal muscles of other shells are attached to a lateral region of the shell in the mantle. The interior mantle muscle is interrupted by two pairs of horizontal buccal muscles, and its attachment partially interrupted by a median muscle to the lip.

In the anterior lip is also a median horizontal muscle attached to I; circular muscles occur about the mouth, and longitudinal muscles from before and behind radiate into the lips.

Muscles of the Radula.

To the description of the other muscles of Chiton may be added a very brief sketch of the muscles of the radula, and of their general direction and places of attachment.

The relations of the radula to the mouth and other organs are best seen in longitudinal sections (*cf.* Fig. 8); the mouth opens into the pharynx, which leads dorsally into the œsophagus (Fig. 8, *oe*); ventral to the œsophagus, the radula sheath opens into the pharynx (at *r*, Fig. 8), and fits around the radula or lingual ribbon, the radula and sheath being extended posteriorly for a long distance, ventral to the œsophagus. On either side of the radula are the so-called "cartilages," long hollow vesicles with thick cartilaginous walls which serve for the attachment of muscles; anteriorly, the radula sheath is

extended laterally on either side, dorsal to the cartilages (*cf.* Figs. 7, 9, 11, *rs*); a pair of glands opens into the œsophagus at a point posterior to these lateral projections (see Fig. 10, *gl*).

The names used for the muscles in the following description indicate in most cases the position and directions of the muscles rather than their function, as that is not understood; the plan is that adopted by Geddes, and in cases where the muscles in Chiton appear to correspond with those in the forms described by him, I have used the same lettering.

The muscles pass from the radula sheath to the cartilage (*ms*$_1$ and *ms*$_2$ of figures); from the shells to the cartilage (*pr*, *cr*, and *lpr* of figures), or to the radula sheath (*or* and *dr* of figures); from the muscular head-fold or foot to the cartilage (*vpr*, *al*, *pl* of figures); and in one case from one cartilage to the other (*vtr*).

The muscles from the radula sheath to the cartilage are of two kinds: broad, flat muscles from the lateral extension of the radula sheath (Figs. 7, 9, *ms*$_1$) running posteriorly to the outside of the cartilage (Fig. 9, *ms*$_1$), and thread-like muscles (approximately circular in cross-section, Figs. 11, 12, *ms*$_2$), running from the part of the radula sheath that immediately surrounds the radula (Fig. 9, *ms*$_2$), posteriorly, to be attached to the inner and dorsal surface of the cartilage (*ms*$_2$, posterior to Fig. 10); these thread-like muscles are attached to the cartilage more posteriorly than the broad muscles, and so cover them dorsally posterior to the lateral projection of the radula sheath (Fig. 11, *ms*$_2$).

The horizontal muscles ("protractors") that are attached in two groups on either side to the anterior part of shell I, and pass between the groups of dorso-ventral fibres (as described in the account of the muscles to the head-fold under I), unite posteriorly into one group, and then pass to the outside of the cartilage, to be attached to it posterior to the attachment of the broad muscles (*ms*$_1$) of the sheath (*cf.* Figs. 7, 9, 10, 11, *pr*). A pair of muscles (Fig. 11, *cr*), attached to I at a point almost as anterior as the attachments of the protractors, cross in the middle line, and pass with the protractors to the cartilages (Fig. 9, *cr*), but are attached to the cartilage anterior to the broad sheath muscles (*ms*$_1$).

The lateral protractor muscles from shell II are attached to the outside of the cartilage, posterior to the attachment of the longitudinal protractors from I (Fig. 11, *lpr*).

The oblique radula muscle from II, that crosses the pedal muscles with the lateral protractor, is attached to the lateral projection of the radula sheath (Figs. 9, 11, *or*).

A group of dorsal muscles (Figs. 10, 11, *dr*) on either side passes obliquely forward from shell II (anterior to the opening of the gland into the œsophagus), to be attached to the portion of the radula sheath that is immediately posterior to the region where the thread-like muscles of the sheath (ms_2) are attached to it. It is these muscles that have been described as compressing the median dorsal shell muscle under II.

Immediately ventral to the attachment to the shell of these oblique muscles from II, are muscles from the floor of the body cavity, in the median line, that pass posteriorly and laterally (Figs. 10, 12, *vpr*), to be attached to the cartilage posterior to the attachment of the thread-like muscles (ms_2) from the radula sheath.

The lateral portion of each cartilage is bound to the muscular head-fold by a large muscle on either side of the posterior limit of the mouth (Figs. 9, 12, *al*). A similar muscle occurs more posteriorly under the edge of the apophysis of III, running from the muscular mass that bounds the body cavity laterally, to the lateral portion of the buccal muscles and cartilage (Figs. 10, 12, *pl*).

The anterior portions of the cartilages of the opposite sides are joined to one another ventrally by a thick transverse muscle (Figs. 7, 9, 12, *vtr*). Finally, on the dorsal side of the buccal mass is a Y-shaped muscle (Fig. 11, and in cross-section, Figs. 7, 9, 10, *y*) attached to the radula sheath.

Historical.

The earliest account which I have found of the musculature of Chiton is the description by Poli in his work on the "Mollusca of the Sicilies," published in 1791. His first figure represents the animal from the ventral side after the removal

of the internal organs, somewhat as in Fig. 3. The oblique dorsal muscles he describes; the pedal muscles have apparently not been cut away close to their attachments, and Poli therefore finds a series of "pyramidal muscles." These are evidently the internal fibres of the latero-pedal muscles seen from the ventral side, for, by comparing cross-sections with Fig. 3, it will be seen that, if in Fig. 3 the latero-pedal muscles had been cut off only to within some distance from their attachment, instead of close to the shell, the fibres of consecutive muscles, as they diverge anteriorly and posteriorly from the shell, would have formed groups pointed toward the median line and spread laterally to meet one another at a level nearer the observer, thus forming pyramids; and there is a pair of pyramidal muscles (the latero-pedal of the anterior and posterior groups) corresponding to each oblique dorsal muscle. Poli describes, outside the pyramids, a circular muscle around the body which is difficult to identify with any one muscle observed, but seems rather to correspond to the muscular tissue of the mantle near the shells. The pyramids, he says, go from the circular muscle to the separate shells and bind them firmly; the shells are still more securely bound by "girding muscles" (apparently the muscles of the cushion) and by the serrations at the edges of the shell; each of these girding muscles, also proceeding from the circular muscle, is attached at its edge to a shell; and the serrations of the shell are deeply imbedded in alveoli, in the circular muscle, as Poli shows in a figure of the dorsal side without the shells. Poli concludes his description of the muscles by showing in a part of the same figure (where the dorsal muscles are cut in the median line and turned back) the "transverse muscles" of the foot that are united together in little bundles.

Middendorff, writing in 1847, gives a very brief account of some of the muscles of Chiton (Cryptochiton) Stelleri; he says the muscles in the body-wall (seen on opening the animal on the dorsal side) are continuations of a flat, muscular sheet, which he calls the "Bauchmuskel"; this muscle bounds ventrally the body cavity, and is the innermost layer of the usual organ of locomotion among the Gastropods, that is, the foot.

Middendorff describes the muscles as running parallel to one another till they reach the sides of the animal, and as then separating into groups, leaving spaces filled with a spongy substance (anterior and posterior groups of pedal muscles with nephridia between). Three parts of the "Bauchmuskel" under each shell partially bound these spaces, and appear to correspond (1) to what I have called the inner fibres of the anterior group, at the attachment to the shell (Poli's pyramidal muscle); (2) perhaps, to the inner fibres of the posterior group, and (3) to the oblique dorsal-shell muscle ; he adds that a longitudinal muscle runs along either side of the dorsal artery, being, therefore, the median dorsal-shell muscle.

Haller marks on one of his figures what he refers to as a longitudinal muscle in the narrow part of the foot, but which I interpret as the fibres of the oblique muscles.

Lang briefly mentions: (1) longitudinal muscles over the foot on either side (the oblique muscles apparently cut transversely in cross-section, as Haller also figures them); (2) muscles in the dorso-ventral direction from the sides of the shell into the foot (latero- and medio-pedal); and (3) the muscles passing in various directions in the foot. The dorso-ventral muscles he regards as the representatives of the shell muscle of Fissurellidae, *etc.*, and of the spindle muscle of the other Gastropods ; he further describes the crossing of the medio-pedal muscles of opposite sides.

The buccal muscles of Cryptochiton were described in detail by Middendorff (1847), and Schiff in 1858 gave a short account of the muscles of the radula in Chiton piceus ; Schiff refers to Middendorf's paper, but leaves his reader to compare the muscles described in the two accounts. Both authors have described the position of the cartilages and radula, the transverse muscles between the cartilages, muscles surrounding the cartilages, and other muscles attached to the radula sheath, to the cartilages, and to the shells and body-wall. Middendorff's figures are not altogether clear, and neither of the authors has studied the muscles in section, so that it is sometimes difficult to understand the exact position of the muscle attachments; in some cases, I have found a different attachment from that

described by Schiff for muscles that otherwise appear to correspond in position with those figured by him. For these reasons I shall not attempt to compare, muscle by muscle, the two descriptions with the present account. The description by Schiff appears to represent more nearly than Middendorff's the condition in the forms that I have studied.

Both Middendorff and Schiff speak of muscles that are attached by both ends to the same cartilage, but I have been unable to find such muscles, and presume from comparison of the drawings, that the muscles from the radula sheath to the cartilage (*ms*$_1$ or *ms*$_2$) are those referred to. The muscles of these groups appear to be attached anteriorly to the cartilage, when seen from the dorsal side; but the lateral projection of the radula sheath, dorsal to the cartilage, produces this effect, and in dissections, as well as in sections, the anterior attachments are seen to be on the sheath.

Both authors have discussed at some length the functions of the muscles, and Middendorff briefly describes some of the muscles of the head region.

Germantown, October, 1894.

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REFERENCE LETTERS.

I Shell I.
II Shell II.[1]
Etc.
II ap apophysis of shell II, *etc.*
A anterior.
aga region of attachment of the anterior group of pedal muscles under IV.
al anterior lateral muscle of the buccal mass.
ao antero-oblique muscle.
ao_1 antero-oblique muscle of the anterior group.
ao_2 antero-oblique muscle of the posterior group.
ao'_1 or ao'_2 antero-oblique muscle (at a low level) of the group next posterior to the group in the section represented.
ap apophysis.
ba branchial artery.
bv branchial vein.
bvn branchio-visceral nerve.
bc body cavity.
bm a portion of the buccal mass protruded through the mouth.
C buccal cartilage.
c cushion of muscles between the shells.
c_1 oblique muscles of the cushion in the transverse plane.
c_2 oblique muscles of the cushion in the sagittal plane.
c_3 oblique muscles of the cushion in the horizontal plane.
cl circular muscles in the lip.
cr crossed muscle of the buccal mass.
cra region of attachment of this muscle.
dpr dorsal protractors of the buccal mass.
dpra region of attachment of these muscles.
dv "dorso-ventral" muscles under I, corresponding to the latero-pedal muscles under the other shells.
dva region of attachment of these muscles.
dv'a region of attachment of the inner group of additional dorso-ventral muscles.
dv''a region of attachment of the outer group of additional dorso-ventral muscles.
gl gland opening into the œsophagus.
F foot.
h horizontal fibres in the transverse plane under I.
HF head-fold of the foot.
hml horizontal median fibres to the anterior lip.
hl horizontal longitudinal muscle from VIII to the foot.
im interior mantle muscle.
ima region of attachment of this muscle.
L lips.
ll lateral longitudinal muscle of the shells.
lp latero-pedal muscle.
lp_1 latero-pedal muscle of the anterior group.
lp_2 latero-pedal muscle of the posterior group.
lpr lateral protractor of the buccal mass.
lpra region of attachment of this muscle.
M mantle.
mc mantle chamber.
md median dorsal muscle of the shells.
mp medio-pedal muscle.
mp_1 medio-pedal muscle of the anterior group.
mp_2 medio-pedal muscle of the posterior group.
ms_1 broad muscles of the radula sheath.

[1] The numerals II and III have been reversed in Fig. 10.

ms$_2$ thread-like muscles of the radula sheath.
o oblique muscle from I and II to the foot.
o$_1$ portion of this muscle from I.
od oblique dorsal muscle of the shells.
oe œsophagus.
oer œsophageal nerve-ring.
oml oblique median muscle to the anterior lip.
omla region of attachment of this muscle.
or oblique radula muscle.
ora region of attachment of this muscle.
P posterior.
pga region of attachment of the posterior groups of pedal muscles under IV.
pl posterior lateral muscle of the buccal mass.
pn pedal nerve.
po postero-oblique muscle.
po' postero-oblique muscle (at a low level) of the group or shell next anterior to the group in the section represented.
pr horizontal protractor muscles of the buccal mass.
pra region of attachment of these muscles.
r radula surrounded by the radula sheath.
r' radula exposed where the radula sheath and œsophagus communicate.
ra radiate muscles to the anterior lip.
rp radiate muscles to the posterior lip.
rs lateral projection of the radula sheath dorsal to the cartilage.
vpr ventral protractors of the buccal mass.
vtr ventral transverse muscles of the cartilages.
y y-shaped muscle of the radula.

EXPLANATION OF PLATE XXXI.

FIG. 1. Outline of shells I, V, and VIII, dorsal surface.

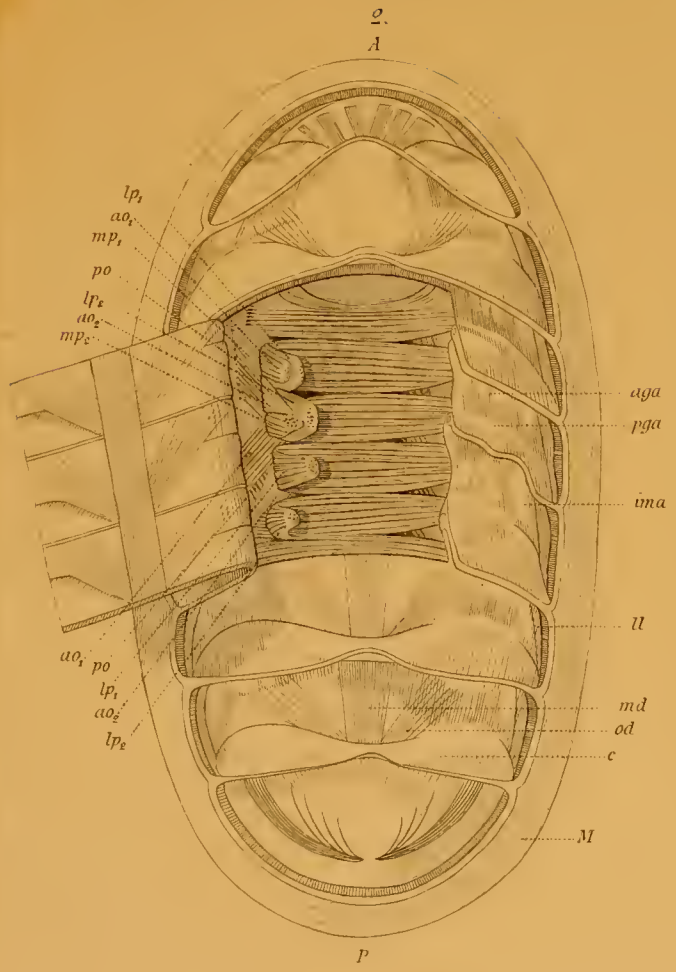
FIG. 2. Chiton viridis, Spengler. Dorsal view after removal of the shells. The shell muscles in the region of shells III (in part), IV, and V have been cut on the right side along the interior limit of the attachment of the pedal muscles, and turned back on the left side; the viscera removed, showing the pedal muscles *in situ* on the right side. The pedal muscles under shells IV and V on the left, are in part dissected near their attachments to the shell, and turned back to show the relations of the muscles to one another. The muscular cushion, dorsal to the right apophysis of V, has been partly removed, to show more clearly the region of attachment of the anterior group of pedal muscles under that shell.

FIG. 3. Diagram of the anterior end of Chiton viridis, Spengler (shells I, II, III, IV, apophyses of V), seen from the ventral side after removal of the viscera and of the pedal and mantle muscles, showing the muscles of the shells and regions of the attachments of the muscles of the foot and mantle and of buccal muscles.

FIG. 11. Diagram of the muscles of the buccal mass (C. viridis, Spengler) as seen from the dorsal side. The dotted lines represent the position of the radula at a lower level, beneath the radula sheath, which, in this region, is extended laterally, dorsal to the cartilages.

FIG. 12. Diagram of the muscles of the buccal mass (C. viridis, Spengler) as seen from the ventral side.

FIG. 13. Diagram showing approximately the relations among the attachments of the pedal muscles to the shells.


2.
A
lp_1
ao_1
mp_1
po
lp_2
ao_2
mp_2
aga
pga
ima
ll
ao_1
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lp_1
ao_2
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md
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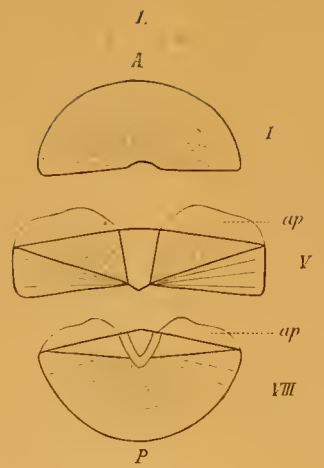
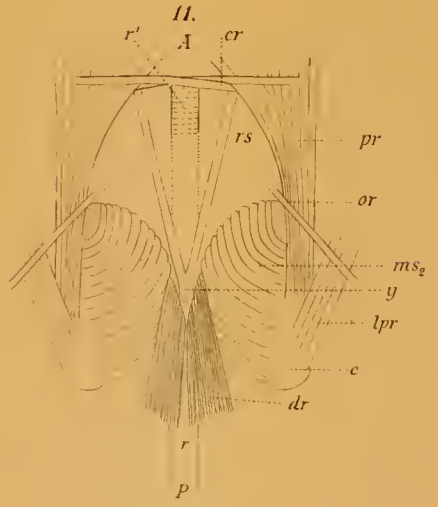
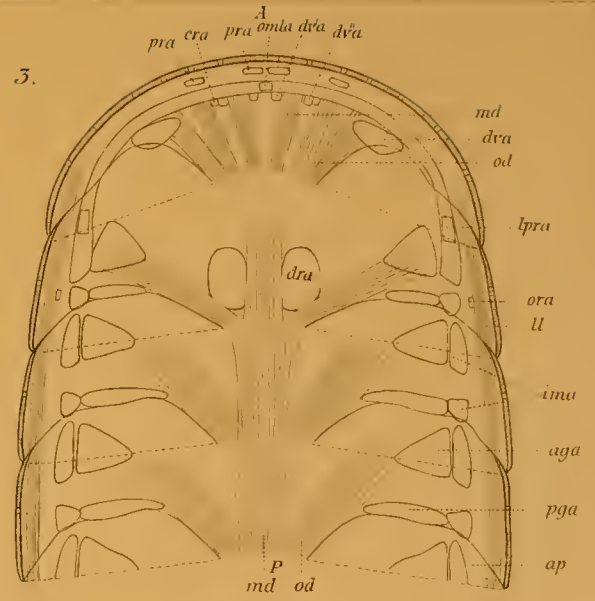
c
M
P


1.
A
I
ap
V
ap
VIII
P


11.
r'
A
cr
rs
pr
or
ms_2
y
lpr
c
dr
r
P


3.
A
pra
cra
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omla
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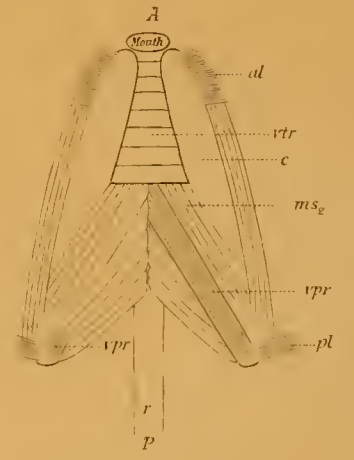
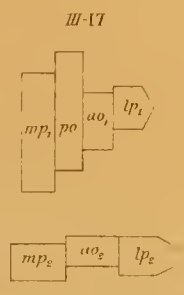
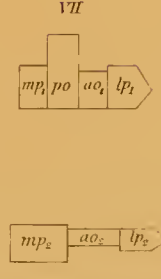
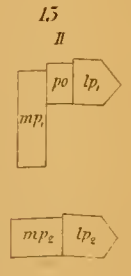
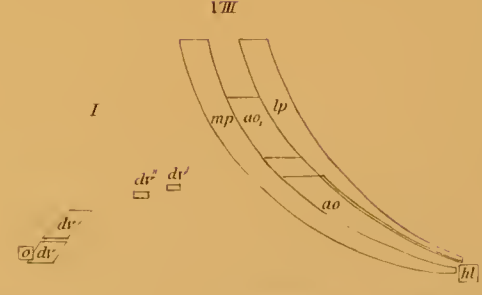

12.
A
Mouth
al
vtr
c
ms_2
vpr
vpr
pl
r
p


III-VI
mp_1
po
ao_1
lp_1
mp_2
ao_2
lp_2


VII
mp_1
po
ao_1
lp_1
mp_2
ao_2
lp_2


13.
II
po
lp_1
mp_1
mp_2
lp_2


I
dr^2
dr^1
dr
o
dr
VIII
mp
ao_1
lp
ao
hl

EXPLANATION OF PLATE XXXII.

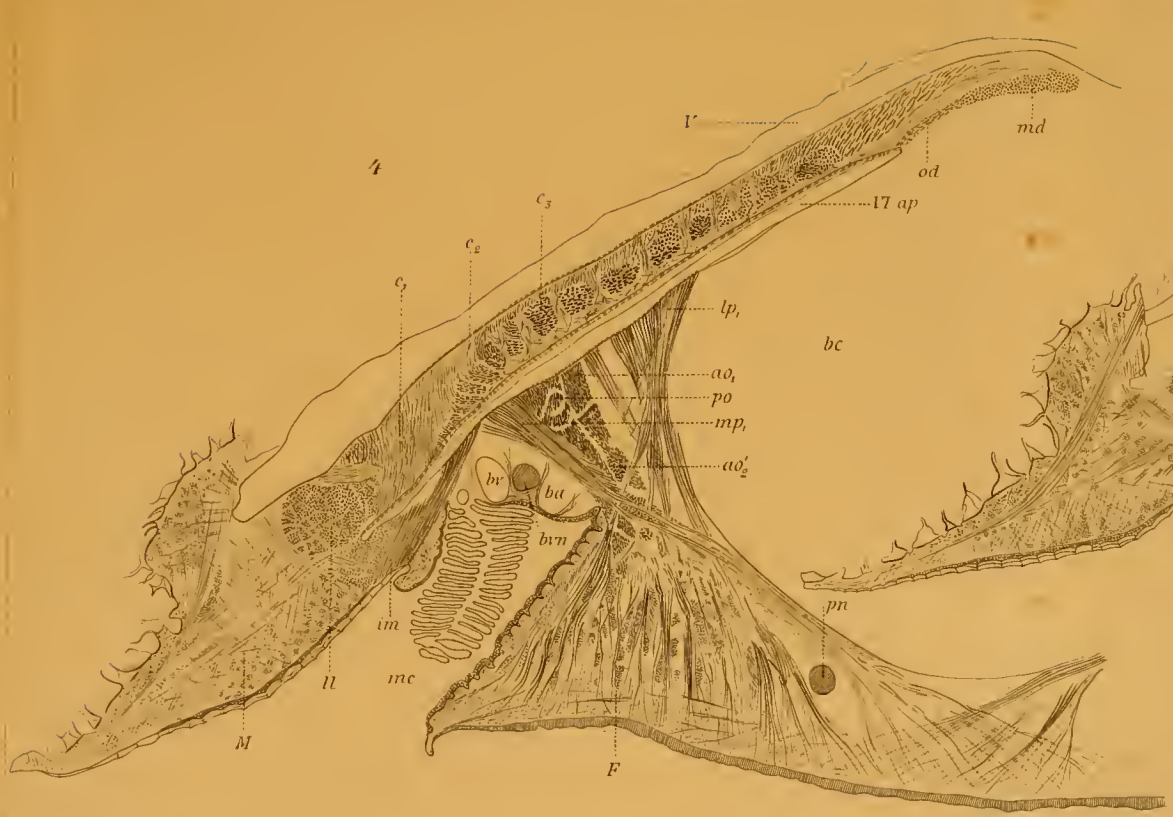
The figures of sections are drawn from preparations of C. olivaceus.

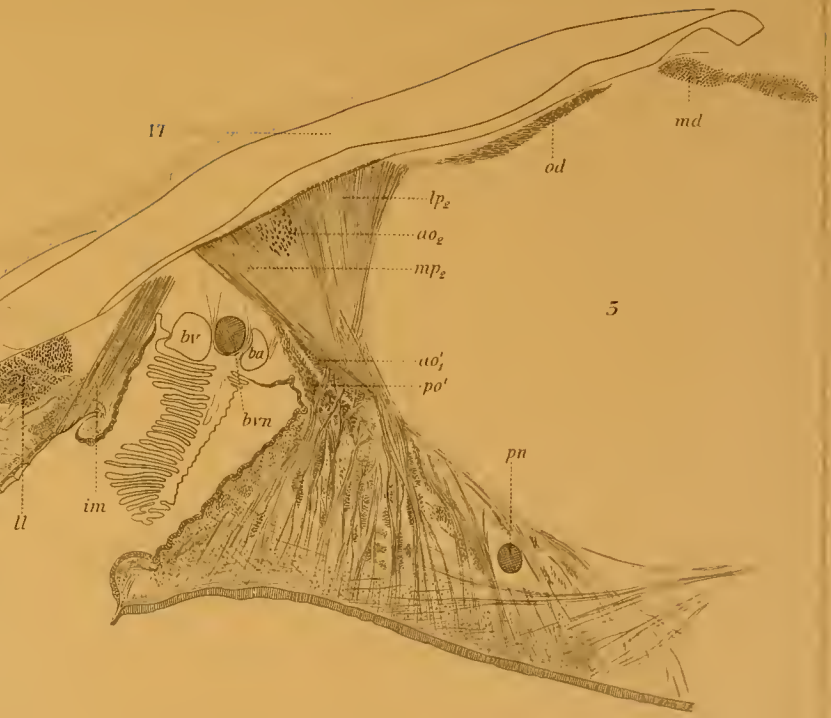
FIG. 4. Cross-section through the anterior group of pedal muscles on one side under VI. Interior mantle muscle (*im*) is cut immediately posterior to the region where a break occurs for blood supply to the mantle. Camera drawing, × 28.

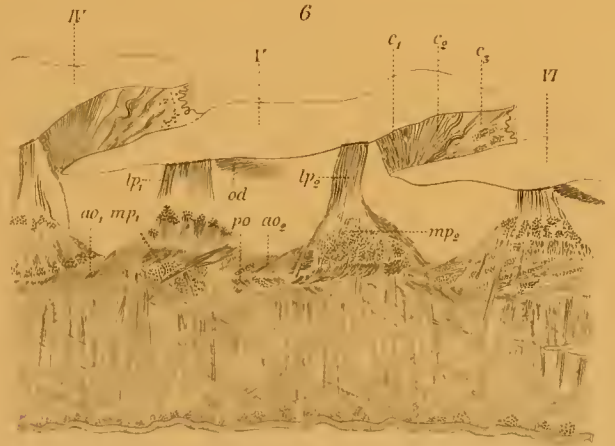
FIG. 5. Cross-section through the posterior group of pedal muscles on one side under VI. Interior mantle muscle (*im*) is cut immediately anterior to the region where the main blood supply passes to the mantle. Camera drawing, × 28.

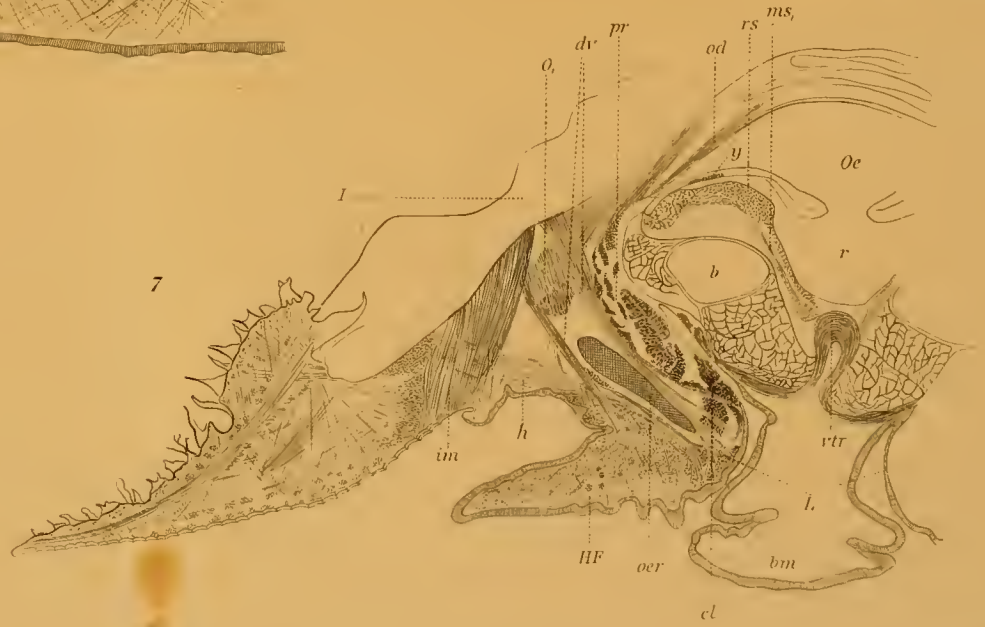
FIG. 6. Sagittal section through the attachments of the latero-pedal muscles of the anterior and posterior groups under shell V; showing also the posterior group under IV and the anterior group under VI. Camera drawing, × 28.

FIG. 7. Cross-section through one side of the posterior region under I. Camera drawing, × 28.


4
V
md
od
VI ap
c_3
c_2
c_1
lp_1
bc
ao_1
po
mp_1
ao'_2
bv
ba
bvn
im
ll
mc
M
pn
F


VI
md
od
lp_2
ao_2
mp_2
5
bv
ba
ao'_1
po'
bvn
pn
im
ll


IV
6
V
c_1
c_2
c_3
VI
lp_1
od
lp_2
ao_1, mp_1
po
ao_2
mp_2


7
O_1
dv
pr
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rs
ms_1
y
Oc
I
r
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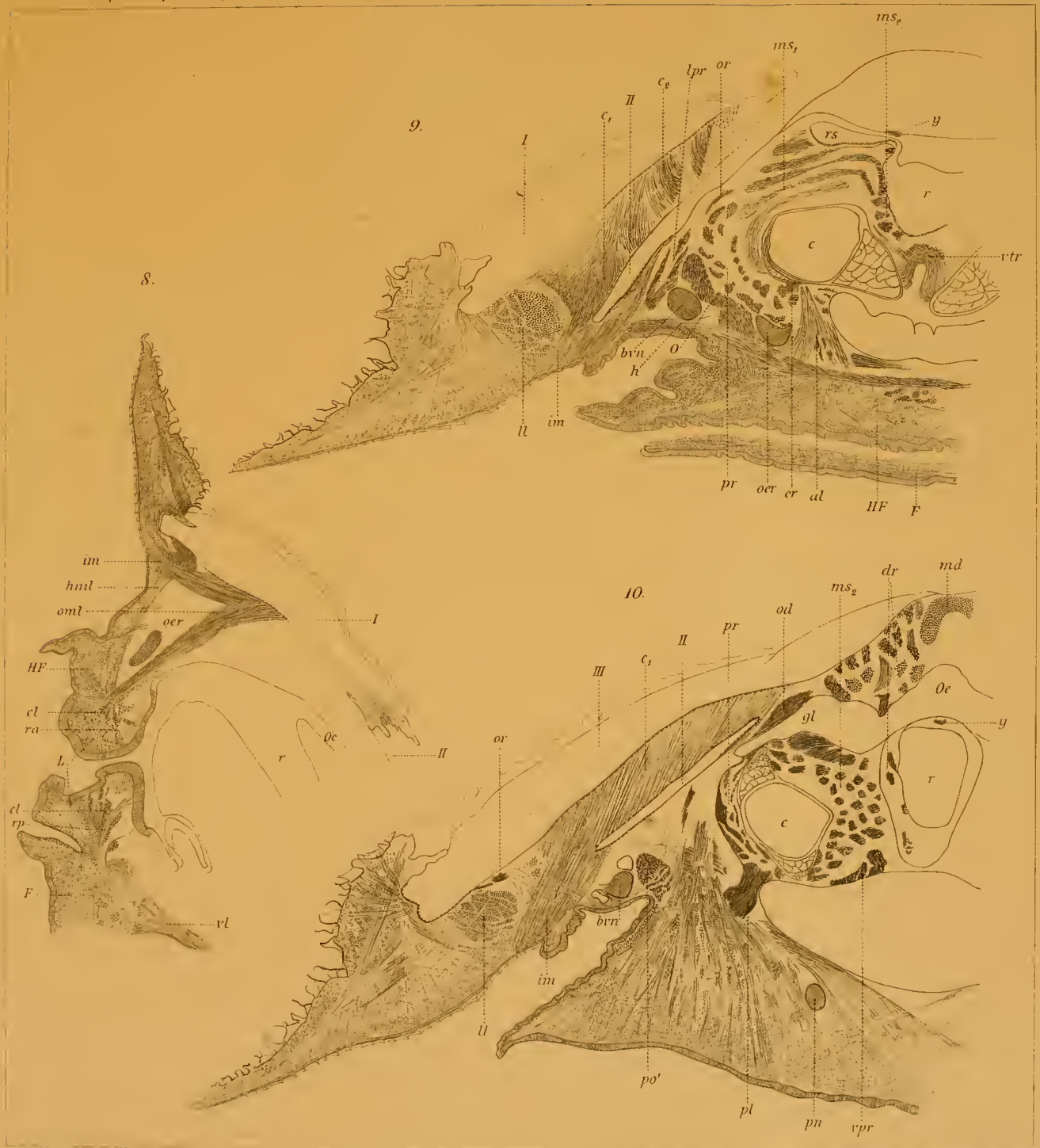
cl

EXPLANATION OF PLATE XXXIII.

FIG. 8. Median sagittal section under I. Camera drawing, × 28.

FIG. 9. Cross-section through buccal mass on one side under II. Camera drawing, × 28.

FIG. 10. Cross-section through buccal mass on one side more posterior than Fig. 9. Camera drawing, × 28.


9.
8.
10.
ms_e
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ms_e
od
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c
bvn
im
ll
po'
pl
pn
vpr

A STUDY OF THE OPERATIVE TREATMENT FOR LOSS OF NERVE SUBSTANCE IN PERIPHERAL NERVES.

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The far-reaching pathological changes which result from loss of continuity in peripheral nerves, and the relative frequency of such injuries, have for many years aroused the attention of physiologists and surgeons. The very voluminous literature bearing on this subject is rich in experimental and clinical observations.

All the evidence, experimental and clinical, goes to show that the function of a divided nerve (peripheral) can be restored if the severed ends are brought together, and retained in close apposition by means of a suture or otherwise ; that a careful coaptation of the divided ends favors return of function ; and that the results are more hopeful if only a short time intervenes between the injury to the nerve and the application of the suture. The underlying principle in all operations for the repair of a divided nerve is, therefore, to obtain as rapid and as accurate approximation of the divided ends as possible.

That return of function may take place even though the divided ends are not carefully adjusted and retained in position, has been shown experimentally and clinically. Surgeons regret the fact, that, after dividing and even resecting a nerve, to alleviate neuralgic pains, the pain may return again, in a longer or shorter time.

Notta, Langenbeck and Hueter, and Weir Mitchell report cases where spontaneous return of function occurred after loss of nerve substance.

In 1827 Tiedemann cut the nerves in the branchial plexus of a dog, and removed 10–12 centimeters. Complete loss of sensation and motion occurred. Within the next two years these were restored, and in 1829, when the dog was killed, the defect was completely healed.

These cases must, however, be looked upon as exceptions, and not considered as disproving the general principle laid down.

In the great majority of cases the surgeon will have no difficulty in bringing the divided ends of a nerve together and applying a suture. The injuries to which peripheral nerves are exposed (among which may be mentioned cuts with sharp instruments, glass or saws, gunshot wounds, etc.) are of such a nature that not much nerve tissue is removed. This is, however, not always the case. In laceration wounds of an extremity several inches of a nerve may be destroyed ; in removing tumors involving nerves the resected ends may be far apart, and again, in cases where inflammation and suppuration follow injury to a nerve, the stump may become so imbedded in a mass of cicatricial tissue that without long and tedious dissection the severed ends cannot be found : at such times the surgeon cannot resort to nerve suture.

A number of methods have from time to time been suggested to obviate the difficulties arising in such cases. To try these methods experimentally, and to establish if possible their comparative value, has been the object of this investigation. Much experimental work dealing with these questions has already been done. A review of this work, with a table giving some clinical cases, will form the first part of this paper. The record of the author's experiments, and the physiological observations made, will be presented in the second part ; and the results of the microscopical examinations in the third part. It is hoped that the subject may be more clearly and completely presented in this form.

Part I. — Review of Literature.

The various methods which have been employed in the treatment of nerve injuries with loss of nerve substance may best be grouped under the following heads : —

(*a*) Nerve stretching (Schüller).

(*b*) Implantation of a nerve segment removed from a recently amputated limb or from one of the lower animals.

(*c*) Tubular sutures (Vanlair).

(*d*) Union of ends with catgut threads, or a bundle of catgut threads (suture à distance, Assaky).

(*e*) Nerve flap from central stump, or a flap from both central and peripheral stumps (autoplasie nerveuse à lambeaux, Létiévant).

(*f*) Grafting of the central end of the peripheral stump of a divided nerve to an accompanying nerve trunk (greffé nerveuse, Létiévant).

(*g*) Cross-suturing the long central and peripheral stump, in cases where two accompanying nerves are cut obliquely, and grafting the central short stump to central long one, and peripheral short stump to peripheral long one (Létiévant, Tillmanns).

(*h*) Resecting the bone or bones in the extremity and suturing the nerve (Löbker).

(*a*) *Nerve Stretching.* — Schüller does not mean to be understood as advising actual stretching of a divided nerve, but states that a nerve can be drawn out from the loose connective tissue surrounding it, and then, by placing the extremity in a position which relaxes the nerve, the separated ends can often be brought together. The force should be applied to the longer end. Kölliker estimates that a separation of 2 to 3 ctm. may in this way be overcome. Schüller has shown that, in some instances at least, the ends may be brought together after a separation of 4 to 5 ctm. He reports the case of a man aged 19, who, nearly six months before Schüller saw him, had injured one of his median nerves under the annular ligament. Sensation in the median area of the hand was lost, and the muscles of the ball of the thumb had atrophied. After exposing the nerve and vivifying the ends, they were 5 ctm. apart. He was able to draw down the central end, and suture it to the peripheral stump. The report records a return of sensation in the part of the hand supplied by the median, and also a return of function in the thumb muscles.

Quite recently Von Hacker reports the case of a boy aged 9, who, about three months before the nerve suture was made, had fallen while carrying a glass pitcher, and had completely divided the left median a little above the elbow. Soon after

the injury the bleeding was controlled, and the wound closed. After the dressing was removed, actual loss of sensation and motion in the hand was observed. At the time of the operation, the ends of the divided median were found to be 4 to 5 ctm. apart. They were drawn out as far as possible, and, after flexing the arm, he was able to bring them together and apply sutures. Function began to return several months after the operation.

It will be noticed that in neither of the above cases was there any *loss of nerve tissue* at the time of the injury. The separation of the ends seems to have been due to their retraction, and probably also to the contraction of the cicatricial tissue of the wound. I may suggest this as a reason why the separated ends could, in these cases, be brought together, while this was not possible in some of the cases where loss of nerve tissue occurred.

This method is by far the simplest and most natural, and should in all cases be tried; care being exercised to use no unnecessary force in attempting to bring the ends together.

(*b*) *Implantation.* — The first attempt at transplantation of a nerve trunk was made as early as 1869. Philippeaux and Vulpian, in a series of experiments, resected the hypoglossal to the extent of 2 ctm. A segment of equal length was removed from the lingual of the same side, and implanted between the ends of the divided hypoglossal, and retained in place by sutures. Their aim seems to have been to determine whether a segment removed from a sensory nerve like the lingual, implanted between the resected ends of a motor nerve (in their experiment, the hypoglossal) would carry afferent impulses. This was tried on seven dogs. In only two of these experiments did the transplanted nerve unite with the resected ends of the hypoglossal. In one of the successful cases, stimulation of the central end excited movements of the tongue, which were more vigorous when the peripheral end was stimulated. On histological examination the transplanted lingual segment was found to consist largely of fibrous tissue and a few medullated nerve fibres. In the other experiment

where union was obtained, the dog lived only twenty-four days, and there was no evidence of regeneration.

This operation was first tried on the human subject in 1878. At this time Albert reported the case of a servant from whom he had removed a sarcoma, about as large as a hazel nut, from the median and with it so much of the nerve that the resected ends were 3 ctm. apart. Before closing the wound, he implanted a segment of a nerve taken from an amputated foot, and sutured the same to the ends of the median. The case was observed for ten days, at the end of which time the wound had healed. No further history is given.

To Glück must be given the credit of again trying implantation of nerves experimentally (apparently without knowledge of any earlier experiments of this nature), and of drawing attention in a number of published articles to the feasibility of this procedure as a legitimate operation in surgical practice.

We are told by Tschirschwitz that Glück, while investigating regeneration of divided nerves, was led to cut a peripheral nerve at two places, and found that, after suturing, regeneration was complete. He next resected a nerve and united the peripheral end of the resected segment to the central nerve stump and *vice versa;* in these experiments the results were also successful. A fowl and rabbit were then chloroformed, and 3 ctm. removed from the sciatic of the fowl, and 3½ ctm. exsected from the same nerve of the rabbit, transplanted, and sutured above and below with silk sutures. On the eleventh day after the operation, the wound, having healed by first intention, was again opened. The union was found to be "ideal"; only by its slight wavy appearance, and by the position of the sutures could the transplanted segment be localized. After isolating the nerve, it was pinched with forceps above the implanted segment and violent muscular movements ensued. The sciatic was then divided above the transplanted piece, and the peripheral end stimulated with the same result. In all, eighteen experiments were made, in some of which the results were equally favorable, in others not so satisfactory.

Glück states, "that a mixed (sensory and motor) implanted nerve unites by first intention, not only when the nerve is

taken from the same, but also when taken from other species; the ends of the implanted segments must, however, be carefully sutured if this result is to be hoped for." Primary union of divided nerves is brought about through the agency of special cells, known, according to Glück, as "specific nerve granulation cells." These are large protoplasmic cells with long processes, and are developed from the nuclei of the sheath of Schwann. They assume a gray-green tinge when stained with osmic acid. The cells anastomose with each other, and unite the central and peripheral end of the divided nerve within the first few days. At the end of six to eight days this investigator notes the finding of young nerve fibres between the spindle-shaped cells. In the course of these young fibres are observed swellings, produced by large nuclei possessing prominent nucleoli, the nuclei resembling the nuclei of the "nerve granulation tissue cells" from which the new fibres are thought to have been developed.

The possibility of union of divided nerves by first intention, *i.e.*, without degeneration of the peripheral end, finds only a few advocates among the many writers who have given thought to this question. Of these, the views enunciated by Glück, based on his interpretation of the experiments above mentioned, are the most outspoken, and most frequently quoted. With him are to be identified Wolberg and Bruch. The former reports one experiment in which primary union took place; this was after partial section of a cat's sciatic. The latter describes one experiment on a kitten where this favorable result was obtained.

The observations of Glück, Wolberg, and Bruch are, however, so at variance with the results obtained by other investigators, that it is difficult to find a suitable explanation for them, or to accept them without further corroboration. In proof of this, I may be allowed to quote from a number of the more recent writings dealing with nerve de- and regeneration.

Bünger states "that every nerve severed from its center undergoes degeneration. The possibility of union by first intention, as upheld by Glück and Wolberg, is for this reason not tenable." Howell and Huber, while admitting Glück's

results as "positive and unmistakable," draw attention to a failure on his part to make a thorough histological examination of the peripheral nerves. In all their experiments, section of a nerve was followed by complete degeneration of the peripheral end.

Stroebe, in a very comprehensive and able research, expresses himself as follows: "Aus allen meinen Experimenten geht hervor, dass die durch Compression von ihrem 'trophischen' Centrum, den Ganglion-Zellen im Central Nervensystem oder Spinal-Ganglion, abgetrennten Nervenfasernabschnitte des Ohrnerven und Ischiadicus jeweils regelmässig der Degeneration anheimfallen, welche sowohl den Axencylinder als die Markscheide ergreifen. Eine Wiedervereinigung der gequetschten Faser an der Druckstelle *per primam intentionem*, welche zu einer physiologischen und morphologischen Restitution der Nerven führen würde, bevor und ohne dass die Degeneration in der peripheren Strecke zur Ausbildung kommt, giebt es nicht."

In Notthaft's article we read: "Nach jeder Verletzung (Verbrennung, Quetschung, Durchschneidung), welche die Nervensubstanz an irgend einer Stelle total zerstört, kommt es zu einer Degeneration des ganzen Peripher von der Verletzungsstelle gelegenen Nervenabschnittes und eines kleineren etwa 1–5 ctm. betragenden Centralen-Stückes. Eine Heilung durch *prima intentio* im Sinne Schiff's giebt es nicht."

The accumulated evidence of nearly all workers in this field coincides with the above statements. In the experiments to be recorded in the second part of this paper, fifty in number, peripheral degeneration followed every nerve section.

Transplantation of nerves has further been tried experimentally by Johnson. His experiments, three in number, were on fowls. In two he resected the sciatic on one side, and transplanted a segment taken from the opposite sciatic. The animals were killed, the one twenty-eight, the other thirty-four days after the operation. In the third experiment he implanted a segment removed from a rabbit's sciatic between the resected ends of a fowl's sciatic. This animal was allowed to live twenty-three days after the operation. In each case

the implanted segment was found to be united to the resected ends of the nerve, but the transplanted portion possessed no conductivity. Tillmanns, in referring to Johnson's experiments, calls attention to the short interval intervening between the operation and the date of the examinations, and correctly assumes that regeneration might have taken place if a longer time had elapsed.

In a series of fifteen experiments recorded by Assaky, dealing with the operative treatment of "nerve defects," transplantation of a nerve was made in four.

(Exp. 3.) A rabbit's sciatic was resected 3 ctm.; a segment 3½ ctm. long, taken from the sciatic of a turkey, was implanted, and sutured above and below with fine catgut. The animal was examined thirty-five days after operation. On pinching the nerve above the implanted segment slight flexion of the foot was observed. The same result when stimulated with the induction coil. On histological examination a few nerve fibres were found in the implanted and peripheral nerve.

(Exp. 12.) The operation was the same as above. The animal was killed sixty-eight days after operation, and the abdominal aorta injected with gelatine and Berlin blue. The operated leg was seized with clonic convulsions. However, these were not as vigorous as on the well side. No new-formed fibres were found in the implanted segment.

(Exp. 14.) The median of a dog was resected 3½ ctm., and a piece of equal length, taken from a rabbit's sciatic, was implanted. The dog was killed thirty-eight days after operation. A cord of fusiform shape was found to unite central and peripheral stump of the median. On cutting into this it was found to contain a "pus-like" substance. No histological examination was made.

(Exp. 15.) Implantation of a segment from rabbit's sciatic between the resected ends of a dog's sciatic. The operation was a failure.

The rapid return of function, obtained by Assaky in the first of the four experiments just quoted, would seem to show that regeneration occurs more speedily in rabbits than in dogs, the animals used by the author in his experiments.

Bünger gives the result of one experiment in which he exsected 1 ctm. from the right sciatic of a dog, and transplanted a segment from the other sciatic. Fifty days after the operation the nerve was examined; at this time he was not able to state what had become of the implanted segment, as in its place were found newly formed nerve fibres. He is, how-

ever, of the opinion that the elements of the transplanted nerve furnished the material for the newly formed fibres. He traces the development of the new axis cylinders from the mitotically increased nerve nuclei and proliferated protoplasm of the sheath of Schwann belonging to the nerve fibres of the implanted segment. Before dissenting from Bünger's interpretation of this experiment, it is necessary and just to explain what his position is concerning degeneration and regeneration of divided nerves. In the first place, he states that there is no time interval separating degeneration and regeneration; regeneration begins soon after the former is initiated, and goes on while degeneration is drawing to a close.

In the guinea pigs used for experimentation the axis cylinder and myelin disintegrate soon after injury to the nerve. At the beginning of the third day the nuclei of the sheath of Schwann begin to divide karyokinetically, and at the same time a proliferation of the protoplasm is noticed. The increase of the nuclei and protoplasm marks the beginning of the regenerative process. This increase continues while the myelin and old axis cylinders are degenerating. Toward the end of the second week the proliferated nuclei begin to arrange themselves in one or two (very seldom more) rows, parallel to the long axis of the nerve; and the protoplasm, which up to this time has been homogeneous, begins to be longitudinally striated. "The fibrillar striation is the anlage of the new axis cylinder, and is developed from the protoplasmic contents of the nerve fibres." This is first observed near the wound. By the end of the second week the new axis cylinders have developed centrifugally some distance away from the seat of injury, and are richly beset with nuclei. In the third week a thin continuous layer of myelin surrounds the axis cylinder. Later another sheath of myelin developing in segments blends with the continuous sheath. The nerve fibres developing in the periphery unite with newly formed fibres, which have their origin in proliferated nuclei and protoplasm of the peripheral end of the nerve fibres in the central nerve stump. These observations were made on degenerating and regenerating nerves hardened in Flemming's solution and stained in safranin, a method

which, with Stroebe, I must condemn as unsuitable for axis cylinder differentiation. I am, furthermore, in accord with this writer in believing that neither Bünger's illustrations nor the description of his section sustain him in his statements. The unequivocal results obtained by the anilin blue-safranin method, as first employed by Stroebe, a stain which gives an axis cylinder differentiation far clearer than when safranin alone is used, leads me to say that the proliferated nuclei of the sheath of Schwann of a degenerating peripheral nerve, or of a degenerating implanted segment, play no part in the development of the axis cylinder. It is an outgrowth of the axis cylinder of the nerve in the central end, as was first clearly shown by Ranvier, later held by Vanlair, Howell and Huber, Stroebe, and Notthaft.

It may further be stated that a number of embryological observations show that the sheath of Schwann and its nuclei, as also the medullary layer, are of mesoblastic origin, while nerve cells and axis cylinders are developed from the epiblast. The axis cylinders are outgrowths from nerve cells ; the naked axis cylinders constitute, at one stage in the development, the entire peripheral nerve. Mesenchymal cells apply themselves to the outside of bundles of naked axes, and grow in between them, and from these the medullary and sheath of Schwann are developed. Minot states "that each cell is the anlage of a medullary segment, the junction of two adjacent cells is the anlage of a node of Ranvier, and the nucleus becomes the internodal nucleus of the sheath of Schwann." (See, also, His, Vignal, and Kölliker.)

In the three experiments of transplantation mentioned by Notthaft only ½ ctm. was removed from the sciatic of a rabbit. In one instance the exsected segment was again inserted ; in the other two a portion was removed from the opposite sciatic, implanted, and fixed with sutures between the resected ends. After fifty days there was regeneration through the implanted piece. Notthaft's experiments can be of little practical importance, as the distance between the resected ends could easily have been overcome by stretching the nerves and applying a suture in the ordinary way.

Quite recently Dr. De Forest Willard, in a communication dealing with nerve sutures, reports fourteen experiments, two of which have to do with implantation.

In one of these ¾ of an inch was removed from the left sciatic of a dog. The segment was reinserted and sutured with fine catgut. Twenty days later the right side was operated upon in the same way. The dog was killed forty-four days after the first operation. On the left side the sciatic was bulbous at the seat of operation, the bulbs being united by a bridge about half the size of the nerve. On the right side there was a thickening of connective tissue which included the graft. In the other experiment ¾ of an inch was removed from the sciatic of a dog, and a segment of nerve ⅞ of an inch was taken from the sciatic of another dog, implanted, and sutured. The dog was killed on the thirtieth day. A small bridge united the divided ends of the sciatic. Physiological and histological examinations, if made, were not reported.

The results obtained in the experiments reviewed may be summed up in the table on the following page.

Excluding the experiments of Glück (for reasons already given), we find only one instance where, after the removal of a segment more than 3 ctm. long, followed by implantation, recovery is reported.

The above table also shows that Glück is the only experimenter that has obtained union by first intention.

The large number of failures and "doubtful" cases would not speak encouragingly for the adoption of this method in surgical practice. That much more favorable results may be hoped for, if the animals are observed for a time long enough to admit of regeneration, I hope to show in the experiments recorded later on.

(*c*) *Tubular Suture.* — Glück experimentally tried implantation of substances other than nerve tissue, using bands of Danish leather, a bundle of catgut threads, strips of muscle and skin, and finally inserting the resected ends of a nerve into a "Neuber's bone drain." In these experiments the implanted substance now and then made "connective tissue union" with the pineurium of the nerve. However, it was usually absorbed or mummified. In no instance did regeneration of the peripheral end occur.

TABLE NO. I.

EXPERIMENTER.	ANIMAL USED.	LENGTH OF NERVE REMOVED.	NO. OF EXPERIMENT.	RESULTS.				REMARKS.
				Union by 1st Intention.	Recovery.	Doubtful.	Failure.	
Philippeaux & Vulpian	Dogs.	About 2 ctm.	7	....	1	....	6	
Glück	Fowls.	About 3 ctm.	18	In some cases.	....	....	....	A full report not given.
Johnson . . .	Fowls.	....	3	....	....	....	3	Animals observed 23–34 days after operation.
Assaky . . .	Rabbits & Dogs.	2–3 ctm.	4	....	1 ?	1	2	
Bünger . . .	Dog.	1 ctm.	1	....	1	....	....	
Notthaft . .	Rabbits.	½ ctm.	3	....	3	....	....	
Willard . . .	Dogs.	¾ in.	2	....	....	2	....	Neither histological nor physiological examination reported.

Soon after Glück's results were made known Vanlair instituted a series of control experiments, using bone drains as a means of uniting the ends of the resected nerve. The sciatic of a young dog was exposed, 3 ctm. removed, and the central and peripheral ends were placed into a decalcified bone tube, and retained in position by catgut sutures. After four months the dog was killed, and the operated nerve laid bare. With the naked eye nothing was seen of the bone tube. The nerve was removed and hardened, and after sectioning, two small fragments of bone were found imbedded in the tissue uniting the resected nerve stump. This tissue contained many new-formed nerve fibres, some of which were even found in the

Haversian canals of the unabsorbed bone. In the peripheral end new fibres were found between the degenerated old sheaths. Other experiments of a similar character have from time to time been reported by Vanlair. He recognizes in "bone drain suture" a group of mechanical condition most favorable for aiding regeneration of nerves after loss of substance, and has gained the conviction that, experimentally, one could reproduce a nerve segment which in length had no limit other than that of the member containing the injured nerve. He was further able to change the course of a nerve by placing the peripheral end of the central stump of a resected sciatic into a bone drain and imbedding the other end of the bone tube in a long, deep incision made in the muscular tissue of the leg, where it was retained by catgut sutures. The wound was then closed. The animal died 6½ months after the operation. Up to that time no return of function had been observed in the leg and foot operated upon. At the post-mortem examination the end of the central sciatic was found bulbous, and from this enlargement could be traced a small cord ending in the muscle; this consisted of nerve fibres, the bundle becoming smaller and the fibres less numerous the further away from the central sciatic the observation was made. Vanlair was able to trace the new fibres 6 ctm. from the bulbous enlargement, and states that he might no doubt have followed them still farther had not the scalpel cut them. The peripheral sciatic was degenerated. This experiment seems to me to offer most convincing evidence that the nerve fibres are an outgrowth from the fibres of the central end; any other explanation seems to me untenable. The peripheral end could in no way take part in the development of the new fibres, and it can hardly be believed that the elements of a decalcified bone tube, or the connective tissue or muscle surrounding it, could furnish any material from which new nerve fibres might have been developed.

Bünger, in two instances, united the resected ends of a dog's sciatic, from which 1 ctm. of nerve tissue had been removed, with a human brachial artery. At the end of forty-three days the space which separated the nerve ends was filled with new fibres. They were arranged in parallel order within the

lumen of the artery, the elastic tissue of which had not been fully absorbed. A number of small branches left the artery through small openings in its wall, and were lost in the surrounding tissue. Before removing the nerves they were tested with an induction current and showed conductivity. Bünger concludes : "Dass die Regeneration grösserer Nervenstrecken nach Resection unter der Bedingung leicht zu Stande kommt, wenn denen von beiden Enden neugebildeten Fasern der Weg und die Richtung gewiesen wird." It is not necessary at this place to again discuss the question whether or not regeneration of the peripheral end from the peripheral nerve fibre can occur. Bünger has, as far as I am able to ascertain, not studied experimentally the fate of the peripheral end after resection. He would have us believe, basing the assumption on observations made by him on degenerating and regenerating nerves after simple section, that, after resection, there is regeneration of the fibres in the peripheral portion from the proliferated protoplasm and nuclei of the degenerating nerves. In four of the author's experiments, where five, ten, twenty-two, and fifty-four days elapsed between the operation and examination, the peripheral stump was in process of degeneration or completely degenerated ; nothing that would point to a regenerative process in the peripheral nerves was observed. I therefore believe that I am justified in hesitating to accept Bünger's conclusions until they receive further corroboration.

The literature bearing on this subject is further enriched by experiments reported by Notthaft and Willard.

The former in two cases exsected ½ ctm. from the sciatic of a rabbit, and united the ends with a segment taken from the aorta of a rabbit. No regeneration was observed after fifty days. The latter resected the sciatic of an old Newfoundland dog for ¾ of an inch, and inserted ⅓ of an inch of the two resected ends into a decalcified bone tube, retaining the same in position by catgut sutures. In ten days the dog was killed, and it was found that the tube had entirely disappeared, and no regeneration had taken place.

The early absorption of an implanted bone tube was also noticed by me in experiments of short duration, and is, of course, an objection to this method. The tubes used in the

author's experiments were decalcified in a one per cent solution of hydrochloric acid, and thoroughly washed in flowing water. It may be that this rapid absorption could in some degree be retarded by decalcifying in chromic acid ; no practical test was, however, made of this.

(*d*) *Suture à Distance.* — Believing that a divided peripheral nerve is regenerated through axis cylinders budding from the central end, and recognizing the fact that it is necessary to make it mechanically possible for the down-growing central fibres of a resected nerve to reach the peripheral end, Assaky tried experimentally to create such suitable mechanical conditions by uniting the ends of a resected nerve with catgut threads, expecting that these would guide the regenerating fibres, and thus aid them in reaching the peripheral part of the nerve. Assaky's experiments were of the following nature :—

In an adult dog he resected 35 millimeters from the left sciatic, and united the ends, which were fixed at a separation of 3 ctm., with four threads of No. 3 catgut. The wound was closed with silver sutures, and a drain was placed into the dependent part of the wound. The dog was killed thirty-five days later. The nerve stumps were found united with a cord somewhat smaller than the nerve itself. After cutting the nerve above the seat of section and rubbing across the distal end, no contractions were noticed. Compression of the nerve excited a single movement. When stimulated electrically, the muscle would contract slowly, "contracting as with regret." Histological examination revealed newly formed fibres in the cord uniting the resected ends, some of which possessed a thin sheath of myelin, others were naked. In the peripheral stump the old degenerated sheaths were found accompanied by fibres newly formed and as yet without a medullary sheath. Six experiments were made, four on dogs, two on rabbits. The length of time intervening between the operation and the examination varied in experiments on the dogs from thirty-five to eighty days, on the rabbits from sixty-seven days to four months. The distance at which the nerve ends were sutured varied from 3 to 1½ ctm.

A review of these experiments shows a favorable termination in each case ; in all, nerve fibres were found in the band uniting the central and peripheral nerve ; conductivity of nerve impulses had been established, as was shown by contraction of the muscle when stimulating the sciatic above the seat of injury, and this after the nerve had been cut some distance above the place of resection.

The rapid return of function obtained in the first of the above four experiments is rather surprising. There would, however, seem to be no reason why Assaky's interpretation should not be accepted. In one of my own experiments, where an ulnar nerve was resected to the extent of 6 ctm., and a "suture à distance" applied, there was no regeneration at the end of thirty-nine days. This may be owing to the fact that in my experiment the down-growing axis cylinders met with greater resistance, as no doubt the connective tissue developing around the catgut threads had an opportunity to become more firmly organized before the developing axes could reach the peripheral end. By way of explanation, I may state that, in all cases of division of a peripheral nerve, the regeneration of the peripheral end depends on the outcome of a struggle between the down-growing axis cylinders and the developing connective tissue between the severed ends, a fact which I hope to show later on in this paper.

This method has further been experimentally tried by Willard, by whom four operations are reported. The results are as follows :—

From the right sciatic of a black pup ¼ inch was removed. The divided ends were stitched with fine chromatized catgut, purposely leaving them ¼ inch apart. After forty-six days the dog was killed. The site of operation upon the nerve was distinguishable only by a slight enlargement, and to the naked eye there apparently had been union. The microscope showed that only one bundle of the nerve trunk had been severed. The ends of the divided portion had separated ½ inch, and were united by fibrous tissue, scattered through which were found many nerve fibres. Judging from the figure (No. 2) given by Willard, the new fibres do not seem to have reached the peripheral end of the nerve. The left side of the same dog was operated upon the day following the first operation ; ¾ of an inch was removed from the sciatic, and a bundle of catgut strands was stitched between the ends. Microscopical examination showed the upper fragment ending in a bulb of fibrous tissue containing numerous nerve fibres, these radiating from the termination of the nerve fibres. The peripheral fragment was degenerated, the new fibres not having penetrated it. (See Fig. 1 of Willard's article, which represents a longitudinal section of the nerve operated upon in the second experiment. The central fibres of the stump are shown in the process of growing down toward the peripheral end; it would seem safe to assume that in time regeneration would have been complete.) In two other experiments of implantation of

a catgut bundle, one was observed fourteen, the other thirty-five days after the operation ; no regeneration was noticed.

No physiological examination was made in any of the above experiments.

(*e*) *Nerve Flaps.* — Létiévant has recommended three operations which may be performed when there is loss of nerve substance. Of these the making of a flap from the central or peripheral stump, or both, is the one more generally known, and is one of the procedures usually mentioned in surgical text-books. With a sharp knife or bistoury the nerve is split for a distance equal to about the length of the nerve segment lost ; the division beginning a short distance from the end of the stump. One of the halves is cut free at its central end (if the flap is made from the central portion), turned down, and stitched to the peripheral stump. Létiévant reports a case where the method was employed (see case No. 17 of table). The simplicity of the method has no doubt done much to win for it approval. It must not, however, be forgotten that it is open to a number of objections. In the first place, assuming that regeneration of the peripheral end takes place through the agency of axis cylinders, which bud from the nerves of the central stump, and remembering that all experimental work shows that it is necessary to create favorable mechanical conditions for these down-growing axes ; I must call attention to the fact that, after turning a flap on its base, the lower end of the nerve fibres of the central segment are not in line with the fibres in the flap, but are rather bent from the course of the nerve at an angle of about 90°, an observation which can easily be made after this portion of the operation has been concluded. The down-growing axis cylinders are, therefore, not guided toward the flap and thence to the peripheral end of the nerve, but bud into the connective tissue surrounding the peripheral end of the central stump, as is clearly shown in longitudinal sections through this region. In the second place, the flap degenerates completely (its connection with the central end in no way retarding this) with a result that we have a condition similar to the one where a nerve is implanted, except that in the latter case a more favorable approximation

with the central end may be attained, and the central nerve segment is not subject to further mutilation.

Three experiments of Willard are interesting in this connection. In these ½ in., ¾ in., and 1½ in., respectively, were removed from the sciatics of three dogs. The ends were split longitudinally, the flaps turned on their base, and united with chromatized catgut. The animals were killed on the tenth, twenty-eighth, and forty-seventh days. The flap and the peripheral part of the nerve were found degenerated. If I understand Willard correctly, regeneration of the peripheral end was not observed, although in the first experiment, the fibres coming from above, separated into a fan-like expansion, but did not reach the peripheral nerves.

(*f*) *Nerve Grafting.* — Létiévant further recommended that the central end of the peripheral segment of a divided nerve, in case it cannot be sutured to the central portion of the same nerve, be fixed by means of sutures to an accompanying nerve. He suggested that the uninjured nerve be denuded of its epineurium at the site of suture. This method can, of course, only give favorable results if, while the epineurium is being removed from the uninjured nerve, some of its fibres are divided. These, in regenerating, we can assume, may grow into the grafted nerve.

As far as I have been able to ascertain, the only experimental observations bearing on this method are reported by Moses Gunn. At his request, Dr. W. H. Sheldon made a series of operations on dogs, which involved the removal of a segment from the ulnar nerve, and the grafting of its peripheral portion to the accompanying median. He states, "that the operations were generally successful, and apparently indicate that through the engrafted connection the parts supplied by the ulnar received their innervation." In another experiment made by Dr. Sheldon, where the ulnar was resected and not grafted, sensation or motion in the parts supplied by this nerve were, after the healing of the wound, in no way impaired. Gunn concludes that this experiment destroys entirely the value of the other.

We are again indebted to Létiévant for the suggestion of cross-suturing the long stump of two divided nerves in cases where the injury is of such a nature that the nerves are cut at a different level. This suggestion admits of adoption only when the injured nerves lie in close proximity, as, for instance, the median and ulnar. That it is possible to obtain return of function after suturing the central end of one nerve to the peripheral end of another, has been shown experimentally by Flourens, Bidder, Gluge and Thiernesse, Philippeaux and Vulpian, Rawa, Gunn, and Howell and Huber. It is evident that only one of the divided nerves can in this way be regenerated, the peripheral end of the other degenerating.

Tillmanns recommends that, after cross-suturing, the short central segment should be grafted on to the long central, and the short peripheral to the long peripheral. This modification does not seem to me to enhance the value of Létiévant's operation. In one experiment by the author, the peripheral short segment was found to be completely degenerated, and the central short stump ended in a large bulb, beyond which no nerve fibres could be traced.

(*g*) *Resecting the Bones in the Extremity, and Suturing the Injured Nerves.* — As a very extreme measure may finally be mentioned a method by Löbker, that of subperiosteal resection of the bone or bones in the extremity to such an extent that the separated nerve stumps can be brought together and sutured. The possibility of want of union between the ends of the resected bones, and the fact that regeneration of the peripheral end has been attained after the employment of measures less formidable, should cause hesitation before this method is determined upon.

Report of Surgical Cases.

Nothing like a complete tabulation of the surgical cases in which a defect in a peripheral nerve was treated by operative means, has, as far as I am aware, been made. Damer Harrison has given us a list of ten cases of nerve implantation. This, I think, is the largest number yet collected.

TABLE NO. II.[1]

No.	Operator and where Reported.	Nerve Operated upon and Nature of Injury.	Primary or Secondary Operation.	Distance Separating Central & Peripheral Segments.	Method used to Overcome the Nerve Defect.	Results.
1	Albert. Einige Operationen an Nerven. *Wiener Med. Presse*, No. 39 und 41, 1885, Jahrgang 26.	Right median in forearm. Result of removing sarcoma of the nerve.	Primary.	3 ctm.	Implantation of a nerve obtained from an amputated foot. Catgut suture above and below.	Patient observed ten days. Wound healed. No further observation.
2	Albert. *Ibid.*	Farmer, age 61. Had enlargements on several nerves, which were supposed to be of sarcomatous nature. June 11, 1881, one of these was removed from right ulnar near the elbow.	Primary.	10 ctm.	Implanted 10 ctm. from posterior tibial taken from an amputated leg. Sutured with catgut.	June 17, 1881. The implanted segment came away in a necrotic state.
3	Kaufmann. Eine Nerven-Transplantation. *Corresp.-Blatt für Schweizer Aerzte.* March 15, 1882. *Revue des Science Médicales.* Tome 24, 1884, p. 305.	Radial nerve was sectioned during operation on necrosed humerus. The nerve was later exposed, the ends vivified. Owing to ankylosis the ends could not be brought together.	Secondary operation.	4 ctm.	Dog's sciatic implanted. Silk suture used.	Operation was followed by fever. The result could not be determined at the time the report was given. No further observation made.

4	Tillmanns. Berlin *Klin. Wochenschrift*, 1885. A short extract of an address delivered in Leipzig in 1884. Boulby, Injuries and Diseases of Nerves, p. 208.	Median and ulnar. Woman, age 22.	Secondary.	4½ ctm.	Nerves from rabbit implanted.	Sensation began to return at the end of four weeks, motion at the end of nine weeks.
5	Gessung. Report by Damer Harrison. Transactions of the Clinical Soc. of London, Vol. 25, 1892.	Median. Removal of Neuroma just above annular ligament.	Primary.	6 ctm.	6 ctm. of the sciatic removed from a rabbit engrafted.	Sensation began to return in two months. Return of muscular movement not recorded.
6	Vogt. Mittheilungen aus der Chirurgischen Klinik zu Greifzwald, Urban, Wien, Leipzig, u. Schwarzenburg, p. 122.	Radial in upper arm. Gunshot wound.	Secondary, 1¼ years after injury.	8–10 ctm.	The defect was bridged by 12 ctm. taken from the two sciatics of a dog.	Suppuration followed. Two months after the operation the nerve did not conduct impulses.

[1] In this table a number of cases referred to in several text-books and in Willard's article are not included. I have not been able to find the original reports, and the accounts given are so brief that I have not thought it desirable to give them a place in the table. The references found are as follows: (1) Dittle made a flap 7 ctm. long, from one of the stumps of an injured ulnar; after prolonged electrical treatment, restoration of sensation and motion is recorded. (2) White replaced the musculo-spiral by the sciatic of a dog; no improvement. (3) Vacetic performed a flap operation on an injured ulnar; suppuration ensued, but the final results were favorable. (4) Hoffman inserted the sciatic of a dog into a radial without result.

TABLE No. II (*continued*).

No.	Operator and where Reported.	Nerve Operated upon and Nature of Injury.	Primary or Secondary Operation.	Distance Separating Central & Peripheral Segments.	Method used to Overcome the Nerve Defect.	Results.
7	Mr. Mayo Robson. Clinical Soc. of London, Friday, June 5, 1889. *Brit. Med. Journal*, Vol. 1, 1889, p. 244.	Girl, age 14. Median, right side just above annular ligament. Patient had a tumor just above annular ligament. This was easily separated from the surrounding cellular tissue, but was inseparably blended with a cord to which it was attached. The cord was severed and tumor removed. After recovering from anaesthetic, patient had complete anaesthesia of the portion of hand supplied by median. Microscopical examination proved the cord to be composed of nerve fibres.	Primary. 48 hours after operation.	2½ in.	Implantation of a posterior tibial nerve which had been removed from a leg amputated by Mr. Ward. The nerve was carried from one amphitheater to another in a warm carbolic acid solution. A segment of suitable length was taken and placed between the ends of the resected median, and sutured above and below by a direct catgut suture.	Thirty-six hours after the implantation, sensations had returned to the extent that touch with a pencil could be localized. Five weeks later the sensation was so perfect that the slightest touch could be localized. At this time there was manifest diminution of the abductor and flexor brevis pollicis; however, they were not completely paralyzed.

8	Dr. H. Landerer. Einheilung eines Kaninchen Nerven in einen Defect des Nerven Radialis. *Deutsche Zeitschrift für Chirurgie*, Vol. 28, 1888, p. 604.	Woman, age 18. Radial nerve. Patient had acute phlegmon of upper arm 1½ years before this operation. ¾ of a year after the beginning of the disease, several sequestra were taken out, the fistula united by incision, and no doubt a segment of the radial removed.	Secondary. More than ½ year after removal of sequestra nerve was exposed.	3½ ctm.	Implantation of rabbit's sciatic. Piece 4½ ctm. in length placed between the ends of radial, not sutured to the nerve, but held in place with several buried catgut sutures.	Wound healed by first intention. Three weeks after implantation electrical stimulation above and below gave contraction of muscles. Methodical electrical treatment was now begun. Ten weeks after operation the patient was shown to the Leipzig Medical Association, and at that time could elevate the hand to the horizontal position even against some pressure. Landerer adds: "Diese Thatsachen scheinen mir eine andere Deutung kaum zuzulassen, als dass in der That das fremde Schaltstück wenigstens für einige Zeit erhalten blieb und nervöse Erregungen zu leiten vermochte. Später wird es natürlich von eigenen Nerven-Fasern durchbrochen."

TABLE NO. II (*continued*).

No.	OPERATOR AND WHERE REPORTED.	NERVE OPERATED UPON AND NATURE OF INJURY.	PRIMARY OR SECONDARY OPERATION.	DISTANCE SEPARATING CENTRAL & PERIPHERAL SEGMENTS.	METHOD USED TO OVERCOME THE NERVE DEFECT.	RESULTS.
9	Mr. Ward. Remarks on Nerve Grafting. By Edw. Atkinson. *British Medical Journal*, Sept. 13, 1890, Vol. 2, p. 624.	Laborer, age 42. Median nerve. July, 1888, patient was operated upon for painful tumor just above internal condyle of right arm. On cutting down, tumor was found to be surrounded by median, out of which it could easily be shelled. November, 1888, patient returned with similar growth in the same location. Jan. 1, 1889, this with 1¾ in. of the nerve was removed.	Primary.	1¾ in.	2½ in. of a median nerve taken from an amputated arm was implanted and sutured with fine catgut.	January 12. Numbness and tingling over the whole limb. January 20. Wound healed. February 2. Slight thickening along the course of the nerve; no pains. Stated that fingers are cold but feel warm. Thumb and index finger very weak; hand looks wasted, especially the thenar muscles. July 26, 1890. Slight degree of wasting of the forearm. Thenar eminence almost entirely absent. Sensation normal to wrist; below, dull over the median area. Movement of index and middle fingers normal in range, though weak. Movements of thumb limited to flexion, extension, and abduction; adduction and opposition entirely wanting.

10	Mr. Mayo Robson. Remarks on Nerve Grafting. By Edw. Atkinson. *British Medical Journal*, Sept. 13, 1890, Vol. 2, p. 624.	Ulnar and median. Silk dresser, age 29. 7 months before seen, patient fell on a scythe and received a deep cut on inner and lower part of arm. At the time of admittance there was loss of sensation and motion in parts below injury supplied by median and ulnar.	Secondary. About 7 mo. after injury, Jan. 30, 1890.	Ends of median 2 in. apart after vivifying stump.	Ulnar nerve was sutured. Internal cutaneous (?) was also found divided, and was sutured to the ulnar. 2 in. of the spinal chord of a rabbit implanted between the ends of median, and held in place by sutures.	February 7. Patient can feel the scratch of a pin on flexor aspect of first phalanx of thumb, also at the root of index finger. Can also tell when a hair located on the first phalanges of little and ring fingers is pulled, but cannot feel the scratch of a pin in these regions. March 4. Sensation in the palmar surface of hand supplied by median, and creeping along the fingers. No marked improvement in ulnar region. April 1. Flexors of forearm all respond to the kathodic closing current. Muscles of the thenar eminence do not react to faradization. April 30. Sensation returning on the back of ring finger. Voluntary flexion of wrist and fingers beginning to be possible. June 13. Nutrition of hand much improved. Patient can pick up small objects. No sensation in area supplied by ulnar.

TABLE No. II (*continued*).

No.	Operator and where Reported.	Nerve Operated upon and Nature of Injury.	Primary or Secondary Operation.	Distance Separating Central & Peripheral Segments.	Method used to Overcome the Nerve Defect.	Results.
11	Edward Atkinson. *Ibid.*	Sciatic nerve. During operation for gluteal abscess 1 in. of the sciatic was removed.	Primary.	1 in.	Immediately the excised portion of the sciatic was again implanted and sutured in place with catgut sutures.	Complete anaesthesia of the limb the day after the operation. Five days later sensation began to return in the toes. Sixteen days after the operation the child could localize the touch of a pin-point anywhere on the limb.
12	Edward Atkinson. *Loc. cit.*	Miner, age 22. Ulnar nerve. Laceration wound of right forearm. Ulnar artery and nerve, flex. sub. dig., flex. carp, ul., and palmaris long. were torn. Ends of ulnar free in the wound.	Primary.	2 in.	2½ in. taken from the two sciatics of a rabbit implanted and sutured with a single suture above and below to the ulnar. Muscles sutured and wound closed.	Five days after the operation, on removing the dressing, the skin flap was under great strain; a stitch was removed, allowing the escape of a large quantity of a brownish, blood-stained pus. The wound was irrigated, and salicylic wool applied. Eighteen days. Sensation beginning to return in ring and little finger. Twenty-five days. Wound closing. Sensation increasing; some return of muscular power is noticed. July 24, three months after operation. Sensation complete in all fingers, except the last two phalanges of little finger. All fingers except little finger can be flexed on the palm.

13	Damer Harrison. A case of Nerve Grafting. Transactions of Clin. Soc. of London, Vol. 25, 1892.	Boy, age 13. Median. Eleven weeks before admission a large glass plate fell on his wrist and divided all the tendons except flex. carp. ul., and the median nerve. At that time the structures were sutured; suppuration and sloughing ensuing. At the time of admission the hand was cold, skin glossy, and muscles atrophied. Flexor tendons were matted together. There was loss of sensation and motion in the median area.	Secondary. Eleven weeks.	2 in.	2 in. were taken from the sciatic of a kitten and sutured between the ends of median with catgut. Fingers were straightened and flexor tendons separated.	Wound healed by first intention. Forty-eight hours after operation prick of a pin could be felt in the palm of hand and over the first phalanx of thumb. Three days, pricking felt over the thumb and first phalanx of index and middle fingers. Nutrition improved at the end of 2½–3 months. Eight months after operation he can abduct the thumb and oppose it to index finger. Flexion of fingers on palm is not perfect.
14	Mitchell Banks. A brief report of the case is given by Damer Harrison, *Loc. cit.*	Ulnar nerve. Excision of neuromatous tumor.	Primary.	4 in.	4 in. grafted from the sciatic of a dog.	Sensation is said to have returned at the end of thirty-six hours.

TABLE No. II (*continued*). — BONE TUBE.

No.	Operator and where Reported.	Nerve Operated upon and Nature of Injury.	Primary or Secondary Operation.	Distance Separating Central & Peripheral Segments.	Method used to Overcome the Nerve Defect.	Results.
15	Socin. Jahresbericht der chirurgischen Klinik zu Basel, über das Jahr, 1888. Also Kölliker, Die Verletzungen und chir. Erkranken der periph Nerven, p. 46, from whom this report is taken.	Ulnar and median of right forearm. Five months before operation nerves were cut. Large "neuroma" found on the central end of the nerves.	Secondary.	4–5 ctm.	The ends of the median and ulnar were united with bone drain. The ends of the nerves were retained in the bone tube with catgut sutures.	Six to eight weeks after the operation, the trophic changes in the hand began to improve. Pronation is beginning to return. In November, the fingers could be flexed a little; "eine Spur." Patient cannot hold anything with the hand.
		SUTURE À DISTANCE.				
16	Glück. Reported by Tschirschwitz in a dissertation, Ueber Nervennaht und Nervenplastik, Berlin, 1892.	Radial, a few ctm. above elbow. August 15, 1887, patient received a stab-wound in arm. Wound was dressed antiseptically. September 4, typical radial paralysis. Nerve was at this time exposed.	Secondary. About three weeks after injury.	6 ctm.	With catgut strands a suture nerveuse à distance was made. Wound closed.	October, 1887, patient went to the electrical clinic of Prof. Bernhardt. June, 1888, patient could extend the closed and opened hand. Can supine the forearm. Adduction and abduction of hand not as perfect as on the well side. April 27, 1892, movements of the hand are normal. A very careful report of the electrical reactions taken from time to time by Bernhardt is given; this is, however, too lengthy to be reproduced in a table of this character.

FLAP OPERATION OF LÉTIÉVANT.

17	Létiévant. Traité des sections nerveuses.	Median and ulnar. Soldier, age 24. In the war of 1871 (Jan. 18) he was struck with a fragment from a cannon ball on the inner side of right upper arm. Median and ulnar were torn and separated a distance of about 5 ctm. A few months after the injury Létiévant saw the case. At this time sensation and motion in the hand were normal. Flexion, extension, adduction, abduction, supination, and pronation could be performed. Patient was treated for several months with electricity. It was at this time observed that the muscles responded to electrical stimulus. March 13, 1872, the nerves were exposed.	Secondary. More than a year after injury.	4 ctm	Flaps were made of the central and peripheral stumps in both median and ulnar, brought together, and sutured with metallic sutures.	Five days after the operation the wound was suppurating; the wound healing by granulation in twenty-one days. Eighty-two days, no improvement. No further report.

TABLE No. II (*continued*). — NERVE FLAPS.

18	Tillmanns. Verhandlungen der Deutschen Gesellschaft für Chirurgie. 14. Congress, 1885, p. 213. This case is also the subject of a dissertation by Oelsner.	Female, age 23. Ulnar and median right side. July 15, 1882, the patient received a cut about two inches above the wrist. Wound suppurated, and finally healed. 3½ months later Tillmanns examined the case, and found sensation and motion in ulnar and median area of hand absent. Hand was atrophied.	Secondary. 4 months after injury.	4½ ctm.	A flap was made of the central and peripheral stumps of both ulnar and median. Flaps were brought together and sutured with two catgut sutures.	Wound healed by first intention fourteen days after injury. From this time, massage, passive movements, and electrical treatment were resorted to. Four weeks after operation an improvement in sensibility was noticed. Nine weeks, began to notice slight degree of motion in parts supplied by ulnar. Fourteen weeks, patient could hold a glass of water and pick up forceps. Sensation was normal with the exception of tips of second and third fingers. One year after operation patient can write with hand, and states she can use it as well as the other. Sensation not yet returned to tips of second and third fingers.

19	Després. Report by Marchant. Gaz. Hebdom., No. 5, 1876. Also by Tillmanns, *loc. cit.*	Patient aged 18. Median in left upper arm. Median, brachial artery and vein were torn. Després tried to suture the ends of the median, but found this impossible.			Després separated the fibres of the ulnar nerve with forceps and placed the end of the median between the separated fibres of the ulnar.	Six days after the operation there was slight return of sensation to middle and index fingers. Four weeks, wound healed to one-fourth its original length. Loss of sensation in last phalanx of middle and index fingers. Movement in hand slow and incomplete. Under electrical treatment the patient received a useful hand. Tillmanns adds that the movements may have been vicarious.
20	Moses Gunn. The Union of Nerves of Different Function Considered in its Pathological and Surgical Relation. Transactions of the American Surgical Association, Vol. 4.	Male, age 36. Right ulnar resected to the extent of ¾ in.	Primary.	¾ in.	Gunn denuded the trunk of the median of its sheath, and placed the broadly champered end of the ulnar upon the denuded surface of median and fastened it with three fine catgut sutures.	Immediately after the operation complete paralysis of sensation and motion was present in the parts supplied by the ulnar. Eighteen days after operation slight return of sensation along ulnar side of ring finger. Three months after operation marked increase of sensibility in the same region, none in the little finger. Four months after operation sensation in ring finger improved, none in little finger. There was increase in muscular power; patient could adduct the hand with considerable vigor, but had no power over the terminal phalanges.

TABLE NO. II (*continued*). — RESECTION OF BONE OR BONES OF EXTREMITY AND SUBSEQUENT NERVE SUTURE.

21	Löbker. *Central-Blatt für Chirurgie*, 1884.	Ulnar and median. 23½ weeks after injury found the flexor tendons, median and ulnar cut.	Secondary.		Resected the bones of the forearm to such extent that the nerves and tendons could be brought together and sutured.	Three months after the operation the patient could hold objects with the hand.
22	Bergmann. A very brief and unsatisfactory report is found in *British Medical Journal*, 1884, Vol. 2, p. 1085.	"One of the nerves of the arm." Loss of motion and sensation in right arm and hand.	Secondary.		Two inches of the humerus resected and ends of the nerve united.	Result not mentioned. (I have been unable to find any report of this case by Bergmann.)
23	Dr. Wheeler. Royal Academy of Medicine in Ireland, Section of Surgery. Report here given is taken from the London *Lancet*, April, 14, 1894, p. 939.	Musculo-spiral. Several months after the injury the ends were found widely separated and could not be brought together.	Secondary.		Portion of the shaft of humerus resected and the ends of the nerve united with sutures.	"Recovery was perfect as regards sensation and motion."

TABLE No. III. — GIVING SUMMARY OF REPORTED CASES.

Operation.	Nerve Operated upon.	Primary or Secondary Operation.	No. of Cases Reported.	Successful.	Improved.	Failures.	No. of Cases Observed Two Months or Longer.	Observed less than Two Months.	Incomplete Report as to Result.	Remarks.
Implantation	Ulnar	Primary	3	....	2	1	1	2	2	Implanted segment necrosed in one case.
		Secondary	....	....	....	....	....	....	....	
	Median	Primary	4	....	3	1	3	1	2	
		Secondary	1	....	1	....	1	....	....	
	Ulnar and median	Primary	....	....	....	....	....	....	....	
		Secondary	2	1	1	....	2	....	....	
	Radial	Primary	....	....	....	....	....	....	....	
		Secondary	3	1	....	2	2	1	1	
	Sciatic	Primary	1	1	....	....	1	....	1	Report gives condition sixteen days after operation.
		Secondary	....	....	....	....	....	....	....	
	Total		14	3	7	4	10	4	6	
Tubular suture	Ulnar and median	Secondary	1	....	1	....	1	....	....	
Suture à distance	Radial	Secondary	1	1	....	....	1	....	....	
Létiévant's flap operation	Ulnar and median	Secondary	2	1	....	1	2	....	....	
Nerve grafting	Median	Primary	1	....	1	....	1	....	....	
	Ulnar	Primary	1	....	1	....	1	....	....	
Resection of bone to the extent of admitting nerve suture	Ulnar and median	Secondary	1	1	....	....	1	....	....	
	Musculo-spiral	Secondary	1	1	....	....	1	....	....	
	One of arm nerves	Secondary	1	?	....	....	?	....	....	
	Grand Total		23	9	9	5	18	5	6	

It is an exceedingly difficult matter to give a correct summary in a table of the above character, the reports at hand often admitting of more than one interpretation. An attempt has been made to give the operator's construction of the results obtained by him, even though this might seem to the writer unwarranted. Unfortunately, in the great majority of the reported cases, sensation and motion do not seem to have been carefully tested; and too little account is often taken of the fact that sensation and even motion is present, at least to some extent, after section of a nerve, in the area to which the injured nerve is distributed. This is admirably shown in case 17 (reported by Létiévant), where, several months after section of the ulnar and median, the sensibility of the hand was not impaired. Weir Mitchell has collected a number of cases, which may be used in corroboration of the above statement. We have further the experiments of Arloing and Tripier, who found that, in order to obtain complete loss of sensation in any of the digits of a dog's foot, it was necessary to cut the four nerve branches distributed in the same. Similar results were obtained by Vanlair after division of the nerves of the posterior extremity. This, of course, makes it difficult to say to what extent the sensibility and muscular contractility, found some time after an operation in the area to which the injured nerve is distributed, should or should not be credited to a regeneration of the divided nerve.

The operation for nerve implantation has been performed fourteen times, eight times as a primary and six times as a secondary operation. Of this number, three are reported as successful, seven as improved, and four as failures. In one of the successful cases, namely, that published by Edward Atkinson, the report covers a period of but sixteen days after the operation, at which time the sensibility was said to be so fully established that the child could localize the prick of a pin anywhere on the limb. This rapid return of sensory function can hardly be attributed to a regeneration of the divided nerve at this early date. Howell and Huber estimate that in man sensation does not return to the peripheral part of a divided nerve until about three months after the operation of suturing

the divided nerve. In Atkinson's case, the impulses were, no doubt, carried along one of the other cutaneous nerves having their distribution in the extremity. The experiments of Vanlair are of interest in this connection. He divided sometimes one, sometimes another, of the nerves in the posterior extremity of dogs, and found that there was very little loss of sensation after section of any one of the nerves.

A closer study of Landerer's case (No. 8) may also be of interest in this connection. In this case more than six months had elapsed since the radial nerve had been injured, and no regeneration had taken place. Three weeks after implanting a rabbit's sciatic, electrical stimulation above and below the injury excited muscular contraction. He assumes that the implanted segment was at least for a time preserved (erhalten), and conveyed impulses ; later it (the implanted segment) may have been replaced by fibres of the radial. Landerer seems to forget that clinical and experimental evidence would justify the assumption that, in the above case, the peripheral portion of the radial was in a state of complete degeneration at the time of the implantation, so that impulses conveyed through the implanted segment could not have reached the muscles. Then, again, all experiments bearing on this subject, except those reported by Glück, show that the implanted portion always degenerates, and even more rapidly than does the peripheral end of a divided nerve ; so it could not have carried impulses. Clinical records show that as a rule the return of motion takes place more slowly than the sensory function. Landerer's conclusions would, therefore, hardly stand critical investigation. The muscular contraction observed on electrical stimulation may have been due to a direct stimulation of the muscles, or to stimulation of some of the other nerves in the arm ; any other explanation does not suggest itself to me.

In a number of cases reported as improved, sensation alone was reëstablished ; the cases either having been dismissed before voluntary muscular movement was observed, or no mention is made concerning its return.

The rapid return of sensation in cases Nos. 7 and 14 would seem to show that the impulses must have been conveyed along

some other path than the injured nerve (in the first the median and in the second the ulnar). The reported cases, where, after section of one or even two of the arm nerves, sensation was not at all or only partially impaired, would lend credence to such a statement.

Of the four cases classed as failures, two (Nos. 1 and 2) are reported by Albert. In one of these, the case was dismissed ten days after the operation ; in the other the implanted segment came away in a necrosed state. In Kaufmann's case (No. 3) the operation was followed by fever, probably due to suppuration. The case was not observed for a time long enough to admit of regeneration of the peripheral end. In Vogt's case (No. 6) the implantation was made 15 months after the injury to the radial, and, after vivifying the ends, they were 12 ctm. apart. The operation was followed by suppuration. The fate of the implanted nerve is not noted ; its connection with the radial may have been lost. The report extends only two months after the implantation, which, judging from the results obtained in other operations on injured nerves, is not long enough to admit of regeneration.

Of the reported cases, the percentage of recoveries is nearly as large for secondary as for primary operations. No attempt is here made to give the average time required for the return of sensation and motion after implantation. The small number of cases at my disposal did not seem to me large enough to warrant such an attempt.

Very little need here be added to what has already been given in the table concerning the other nine cases in which operative means other than implantation were resorted to. I would, however, beg indulgence for again referring to the two cases of nerve grafting (Nos. 19 and 20) reported by Desprès and Gunn, as their interpretation of the results obtained seems to me wholly unwarranted. In Desprès' cases the peripheral median was grafted on to the ulnar ; the wound does not seem to have healed by first intention. Six days after the operation there was some return of sensation to the middle and index fingers, and, at the end of four weeks, the sensibility in these fingers over the two proximal phalanges would seem to be

normal, while it was absent in the distal phalanges. It will be remembered that the thumb, index, and middle fingers are supplied conjointly by the median and radial. A return of sensation in these fingers would therefore not give conclusive evidence of median regeneration, as the impulses may have been carried along the radial. Weir Mitchell long ago drew attention to the fact that the index and middle fingers may be flexed and the thumb opposed, to some extent at least, by muscles not supplied by the median.

In lieu of the above facts, Tillmanns would seem to express himself correctly, when he states that in the above case the "Motilität" may have been vicarious.

Gunn's case involved the grafting of a resected ulnar to the accompanying median. In this case there was loss of sensation and motion in the ulnar region of the hand. Eighteen days after the operation, there was a slight return of the sensibility along the ulnar side of ring finger, which gradually increased, so that four months after the operation it was quite normal; there, was, however, no sensation in the little finger. If we recall the distribution of the ulnar in the hand, it will be remembered that the ring finger is supplied by this nerve only on the ulnar side, the radial side receiving its innervation from both the median and the radial, while the little finger has no other nerve supply. The sensory impulses coming from the ulnar side of the ring finger may, therefore, have been carried along the median or radial. The results of Arloing and Tripier, already quoted, show that this would be the case for dogs. It is important to notice that there was no return of sensibility in the little finger, a fact not explainable if the return of sensation to the ulnar side of the ring finger is attributed to ulnar regeneration. The ring and little fingers could not be flexed at any time after the operation, although the flex. carp. ul. seemed to contract. If the explanation given above is correct, the evidence in both of these cases would show that regeneration of the peripheral portion of the divided nerve had not taken place through the engrafted connection.

The conclusions drawn from data obtained from the experimental work reviewed, the clinical cases reported, and from the

results obtained in my own experiments will be presented at the end of this paper.

Part II. — Record of Experiments and Physiological Examinations.

As has been previously stated, the aim of this research has been, — to try, experimentally, the various methods suggested for repairing loss of substance in peripheral nerves, when such loss is so great that the ends of the divided nerve cannot be brought together by the ordinary methods of nerve suture ; to find out, if possible, which one of the many methods used is likely to give the most favorable result ; to obtain some idea of the time required for the return of function to the peripheral end ; and to establish the structural changes which take place during degeneration and regeneration. The experimental work has, therefore, been partly physiological and partly histological. The record of the experiments and the physiological observations made will form one part of this paper, and the results obtained from microscopical examination of the preparations will be recorded in another part.

The animals used for operations were dogs. With but few exceptions the ulnar nerve was selected for experimentation ; in some few instances the median or sciatic was used. At the time of operation and examination the animals were narcotized with large doses of morphia sulphate injected hypodermically in the inguinal region, and, if thought necessary, this was followed by ether. Antiseptic precautions were observed with as much detail as can well be indulged in when the operations have to be performed in a general laboratory. The hair over the course of the nerve selected for the operation and the surrounding parts was removed with a razor, and the skin thoroughly washed, first, with soap and water, and then with a five per cent carbolic acid solution. The animal was then covered with towels sterilized by steam. The instruments and sponges were sterilized in the same way, and while operating the instruments were kept in a two and one-half per cent carbolic acid solution, and the sponges in a $\frac{1}{2000}$ bichloride of mercury

solution. The catgut used for suturing the nerve was sterilized for thirty minutes in boiling alcohol.

In each case the wound was closed by a double set of sutures. The subcutaneous connective tissue, which had been divided in cutting down to the nerve, was brought together over the sutured nerve by means of four to six chromatized catgut sutures. The edges of the wound in the epidermis were then united with braided silk and covered with iodoform. It was hoped that, even though the "skin wound" might open in a few days, the connective tissue which formed a covering for the nerve would, in the meantime, have united, and thus retain in place the implanted substance; this was found to be the case. Now and then several of the silk sutures gave way, but in no instance were the ends of the nerve stumps nor the implanted portion exposed. In those experiments where a segment of a nerve was implanted, this was, in all but two cases, taken from the sciatic of a cat. While the nerve to be sutured in the dog was being exposed and resected, an assistant chloroformed a cat, removed the skin from the posterior surface of one of its hind legs, and then thoroughly washed the denuded surface with a five per cent carbolic acid solution. The sciatic was then exposed, exsected, and transferred to the wound in the dog's arm. No attempt was made to stitch the central end of the segment taken from the cat's sciatic to the central stump of the dog's ulnar. The manner of preparing the bone tube and the bundle of catgut threads, implanted in a number of cases, will be described in detail when such operations are mentioned. The irritability of the nerves was tested at the time of examination with induction shocks from a Du Bois Reymond coil, the ordinary wire electrodes being used. The strength of stimulus necessary to produce reflexes or excite contractions of muscles supplied by the nerve under examination was estimated by the distance separating the primary and secondary coil. In all examinations the same battery, induction coil, and electrodes were used, so that the results obtained in the various experiments admit of comparison. It is, of course, understood that much reliance cannot be placed on such comparisons, as the strength of the shocks

TABLE NO. IV. — TABULAR SYNOPSIS OF OPERATIONS AND RESULTS OBTAINED.

No. of Experiment.	Animal Operated upon.	Nerve Operated upon.	Extent of Nerve Tissue Removed.	Manner of Bridging the Nerve Defect.	Time between Operation and Examination.	Result of Physiological Examination.
1	Full-grown hound.	Ulnar R. S.	6 ctm.	Implantation of cat's sciatic.	2 days.	Peripheral ulnar not yet degenerated.
2	Do.	Ulnar L. S.	Do.	Do.	3 days.	Do.
3	Young black spaniel.	Ulnar R. S.	Do.	Do.	Do.	Muscles contract on cutting peripheral ulnar.
4	Do.	Ulnar L. S.	Do.	Do.	Do.	Do.
5	Small black bitch.	Ulnar R. S.	5 ctm.	Implantation of ulnar nerve from left side of same dog.	4 days.	Muscles contract feebly on cutting peripheral ulnar.
6	Large female mastiff.	Do.	7 ctm.	Implantation of cat's sciatic.	5 days.	No contraction of muscles on cutting peripheral ulnar.
7	Full-grown black spaniel.	Ulnar L. S.	6 ctm.	Do.	6 days.	Irritability of peripheral ulnar lost.
8	Do.	Ulnar R. S.	Do.	Do.	Do.	Do.
9	Small black dog.	Ulnar L. S.	5 ctm.	Do.	9 days.	Do.
10	Large black mongrel.	Ulnar and median R. S.	4 ctm.	Implantation of cat's sciatic, sutured above to ulnar and median, below to ulnar.	Do.	Do.
11	Full-grown black mongrel.	Ulnar L. S.	Do.	Implantation of cat's sciatic.	Do.	Do.
12	Small Scotch terrier.	Ulnar R. S.	7 ctm.	Implantation of ulnar of L. S. of same dog.	10 days.	Do.
13	Small black mongrel.	Do.	5 ctm.	Implantation of cat's sciatic.	12 days.	Do.
14	Yellow and white mongrel.	Ulnar and median R. S.	4 ctm.	Implantation of cat's sciatic, stitched to ulnar and median above, and ulnar below.	21 days.	Do.

15	Yellow and white mongrel.	Ulnar L. S.	Do.	Implantation of cat's sciatic.	Do.	Irritability of peripheral ulnar lost.
16	Small black bulldog.	Ulnar and median R. S.	7 ctm.	Implantation of cat's sciatic, sutured to ulnar and median above, and ulnar below.	39 days.	Sensibility beginning to return to peripheral ulnar just below peripheral wound.
17	White and brown mongrel	Ulnar and median R. S.	8 ctm.	Implantation of cat's sciatic.	117 days.	Return of sensibility and conductivity of motor impulses to upper part of peripheral ulnar.
18	Small white spitz, full grown.	Ulnar R. S.	Do.	Do.	120 days.	No return of irritability of peripheral ulnar.
19	Brown mongrel.	Do.	6 ctm.	Do.	121 days.	Results as in experiment No. 17.
20	Brown and white spaniel.	Ulnar L. S.	7 ctm.	Do.	Do.	Do.
21	Large black mongrel.	Median R. S.	5 ctm.	Do.	136 days.	Complete return of sensibility and conductivity of motor impulses.
22	White bulldog.	Ulnar R. S.	4 ctm.	Do.	149 days.	Do.
23	White and brown mongrel.	Ulnar and median branch, L. S.	8 ctm.	Implantation of cat's sciatic, sutured to ulnar and median above, and ulnar below.	152 days.	Do.
24	Large shepherd dog.	Ulnar R. S.	4 ctm.	Implantation of cat's sciatic.	Do.	Do.
25	Young mastiff, about one year old.	Ulnar R. S.	6 ctm.	Do.	182 days.	Complete return of sensibility and motor conductivity.
26	Black and brown hound.	Do.	7 ctm.	Secondary implantation. Removed 6 ctm. of ulnar, and, 41 days later, after vivifying ends, implanted 7 ctm. of cat's sciatic.	155 days.	Regeneration of motor and sensory fibres in peripheral ulnar to about the middle of forearm.

TABLE NO. IV (*continued*). — TUBULAR SUTURE.

No. of Experiment.	Animal Operated upon.	Nerve Operated upon.	Extent of Nerve Tissue Removed.	Manner of Bridging the Nerve Defect.	Time between Operation and Examination.	Result of Physiological Examination.
27	Young mongrel.	Ulnar L. S.	7 ctm.	Decalcified bone tube.	5 days.	Irritability of peripheral ulnar lost.
28	Full-grown shepherd dog.	Do.	6 ctm.	Do.	10 days.	Do.
29	Large bulldog.	Do.	Do.	Do.	22 days.	Do.
30	White and brown mongrel.	Sciatic L. S.	3 ctm.	Do.	54 days.	Do.
31	Brown and white spaniel.	Ulnar R. S.	6 ctm.	Do.	121 days.	Irritability beginning to return to central end of peripheral ulnar.
32	White bulldog.	Sciatic R. S.	5 ctm.	Do.	130 days.	No return of irritability to peripheral sciatic.
33	Large black mongrel.	Ulnar R. S.	Do.	Do.	136 days.	Scarcely perceptible return of irritability to peripheral ulnar just below bone tube.
34	Black and brown hound.	Ulnar L. S.	7 ctm.	Secondary bone tube implantation. Removed 6 ctm. of left ulnar. 41 days later, after vivifying ends, implanted 8 ctm. of bone tube.	55 days.	Regeneration of motor and sensory fibres to about the middle of the forearm.

Suture à Distance or Catgut Bundle.

35	Young mongrel.	Ulnar R. S.	6 ctm.	A bundle of catgut threads.	5 days.	Irritability of peripheral end lost.
36	Full-grown shepherd dog.	Do.	Do.	Do.	10 days.	Do.
37	Large bulldog.	Do.	Do.	Do.	22 days.	Do.
38	Small black bulldog.	Ulnar L. S.	5 ctm.	Assaky's catgut suture à distance.	39 days.	Do.
39	Brown mongrel.	Do.	Do.	Do.	136 days.	Return of irritability to sensory and motor fibres in peripheral ulnar to 3 in. below wound.
40	Large black mongrel.	Do.	Do.	A bundle of catgut threads.	Do.	No return of function.
41	Shepherd dog.	Do.	4 ctm.	Do.	152 days.	Complete return of function to peripheral ulnar.

Nerve Flaps.

42	Black spaniel.	Ulnar L. S.	5 ctm.	Létiévant's flap operation, flap from central stump.	5 days.	Complete loss of irritability of peripheral ulnar.
43	Do.	Ulnar R. S.	Do.	Do.	Do.	Do.
44	White and brown mongrel.	Do.	Do.	Do.	10 days.	Do.
45	Do.	Ulnar L. S.	Do.	Do.	Do.	Do.
46	Large yel. and bl. mongrel.	Ulnar R. S.	6 ctm.	Do.	64 days.	Do.
47	Full-grown greyhound.	Do.	5 ctm.	Do.	147 days.	No return of function to peripheral ulnar.
48	Do.	Ulnar L. S.	Do.	Létiévant's flap operation, flap from cent. and periph. stump.	Do.	Do.
49	Full-grown white bulldog.	Median and ulnar L. S.	4 ctm.	Létiévant's operation of grafting the peripheral stump of injured nerve to accompanying uninjured nerve trunk.	149 days.	No return of function to peripheral median stump.
50	Young mastiff, about one year old.	Ulnar and median L. S.	Do.	Tillmanns' operation of cross-suturing and grafting.	182 days.	Complete regeneration of peripheral ulnar and degeneration of grafted peripheral median stump.

TABLE No. V. — SUMMARY.

Operation.	No.	No. where 120 Days or More Intervened between Operation and Examination.	Cases Observed 120 Days or Longer.			Failures.
			No. in which Regeneration Was Complete.	No. in which Regeneration Extended to Middle of Forearm and to Muscular Branch of Flexo Carp. Ulnaris.	No. in which Regeneration Extended to just below Peripheral Wound.	
Nerve implantation	26	10	5	4	....	1
Bone tube implantation	8	4	....	1 (Secondary implantation.)	2	1
Implantation of catgut threads . .	7	3	1	1	....	1
Létiévant's flap operation	7	2	....	....	....	2
Létiévant's grafting	1	1	....	....	....	1
Tillmanns' cross-suturing and grafting.	1	1	....	....	....	Cross-suturing successful, grafting a failure.

emanating from a Du Bois Reymond instrument, in which the distance between the primary and secondary coils is ten centimeters, would not, of necessity, be the same at different times even though the same apparatus were used. The distance separating the primary and secondary coils is expressed by the symbol *x ctm. S. C.*

In describing the operations here recorded, the following terms have been made use of to designate the different portions of the nerve operated upon, and its relation to the substance implanted : the central portion of the resected nerve has been called the *central stump ;* the peripheral portion, the *peripheral stump ;* the junction of the central end of the implanted portion and the central stump, the *central wound ;* and the junction of the peripheral end of the implanted substance and the peripheral stump, the *peripheral wound.*

The order of the operations given in the table on pp. 668–72, some of which are more fully described in the following pages, is not chronological. It seemed to the author more logical to base it upon the time intervening between the operation and the examination. In doing this it was found necessary to rearrange the notation of the experiments. Often two experiments were made on one dog, the right and left sides being used in succession. In all, thirty dogs were operated upon.

Record of Operations and Physiological Examinations.

This record gives a full account of only a part of the operations made. As a detailed description of all the experiments would involve some repetition, only those of interest physiologically have been selected. For the remaining experiments the reader is referred to the preceding tabular synopsis.

(*a*) Nerve Implantation.

Experiment 1. — Dec. 9, 1893.

Full-grown hound. Hypodermic injection of $\frac{3}{5}$ grm. morph. sulph., followed by ether.

Operation. — The right ulnar nerve was exposed for a distance of about 8 ctm., and exsected to the extent of 6 ctm. A segment of equal length was removed from the left sciatic of a cat, and placed between the cut stumps of the ulnar. The implanted segment was retained in place by

means of a catgut suture passed through each end of the transplanted segment of the cat's sciatic, and through central and peripheral stumps of the ulnar, in this way bringing the ends of the implanted segment in close apposition with the ends of the ulnar stumps. The divided connective tissue was then closed over the nerve by means of five buried chromatized catgut sutures. The dermis was brought together by means of a continuous silk suture.

Examination. — Dec. 11, 1893 (two days after operation).

The wound was healing by first intention. The central end of the implanted segment was fixed to the peripheral end of the central ulnar stump by an inflammatory newly formed tissue. The catgut could not be seen. The peripheral end of the implanted segment was fixed to the peripheral ulnar by the suture. The electrical test was not recorded. Cutting the peripheral ulnar with scissors produced contraction of the muscle supplied by the ulnar.

The nerve was hardened in Flemming's solution, and stained in safranin and licht grün.

The results of experiments 2, 3, and 4 were essentially the same as in the first experiment.

Experiment 5. — Aug, 10, 1893.

Small black bitch. Hypodermic injection of $\frac{2}{5}$ grm. morph. sulph., followed by ether.

Operation. — The right ulnar was exposed, and resected to the extent of 5 ctm. The left ulnar was then removed to the extent of 5 ctm., and implanted between the ends of the resected right ulnar, and fixed by means of a single direct catgut suture above and below, and the wound closed.

Examination. — Aug. 14, 1893 (four days after operation).

The epidermal wound was open for a short distance, the implanted nerve was imbedded in granulation tissue.

The muscles contracted very feebly on cutting the peripheral ulnar.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 6. — Aug. 10, 1893.

Large female mastiff. Hypodermic injection of $\frac{4}{5}$ grm. morph. sulph., followed by ether.

Operation. — The right ulnar was exposed for 8 ctm., and resected to the extent of 7 ctm. A segment of equal length was transplanted from the left side, and wound closed.

Examination. — Aug. 15, 1893 (five days after operation).

The epidermal stitches had given way. The nerve ends and implanted segment were surrounded by granulation tissue.

No contraction of the muscles occurred on cutting the peripheral ulnar.

The nerve was hardened in Müller's fluid, and sections stained in anilin blue and safranin.

In experiments 6 to 13, inclusive, there was found complete loss of irritability of the peripheral stump of the divided nerve, as shown by mechanical and electrical stimulation.

Experiment 14. — Oct. 23, 1893.

Yellow and white mongrel, narcotized by means of $\frac{3}{5}$ grm. morph. sulph.

Operation. — The right ulnar and median were exposed, and resected to the extent of 4 ctm., and a segment of equal length removed from a cat's sciatic was implanted, stitched above to ulnar and median by a single direct catgut suture, and below to the peripheral ulnar. The subcutaneous tissue was sutured over the nerve by means of five buried catgut sutures, and the epidermis closed by five silk sutures.

Oct. 27. Several silk sutures had given way, and the wound was granulating.

Nov. 14. Wound almost entirely healed. The hair was falling out on the inner side of forearm, and a small ulcer had formed on radial side of the wrist. The sensation in the foot seemed quite normal, and the dog used the foot in walking.

Examination. — Nov. 14, 1893 (twenty-one days after operation).

The implanted nerve and ends of the nerve stump were surrounded by well-formed connective tissue.

Electrical stimulation with induction shocks at 5 ctm. S. C., below implanted segment, showed no contraction, and no evidence of pain. Stimulation above the implanted segment at 5 ctm. S. C., after severing the nerve 5 ctm. above central wound, showed no contraction.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 15. — Oct. 23, 1893.

This experiment was performed on the ulnar of left side of dog used for experiment 14.

The operation, duration of experiment, and the results obtained were the same as in the preceding experiment.

The nerve was hardened and stained as in experiment 14.

Experiment 16. — April 21, 1894.

Small black bulldog about one year old. $\frac{3}{5}$ grm. morph. sulph. was injected.

Operation. — The ulnar and median were resected to the extent of 7 ctm., and 7 ctm. of a cat's sciatic implanted and sutured, above to ulnar and median with single direct catgut sutures, below to peripheral ulnar with same sutures. Wound closed.

Wound healed by first intention. At no time did the dog seem to have any loss of sensation or motion in the operated leg or foot.

Examination. — May 28, 1894 (thirty-nine days after operation).

Wound completely healed. On exposing the nerves the central stump of the ulnar and median was found to terminate in a large bulb, from which a cord, much smaller in cross-section than the central nerve stump, could be traced to the peripheral ulnar. The central bulb, the implanted portion, and the central end of the peripheral stump of ulnar were firmly imbedded in fibrous tissue.

Electrical stimulation of the peripheral ulnar with induction shocks at 5 ctm. S. C. (after all the other nerves in the arm above the elbow had been cut) showed no contraction of the muscles in the forearm. The presence of feeble reflexes led to the inference that there was some return of sensibility in the peripheral ulnar just below the peripheral wound. Stimulation of the ulnar above the implanted segment after the ulnar had been severed from the center showed no contraction at 5 ctm. S. C.

Cutting the ulnar above and below the implanted segment produced no contraction.

The nerve was hardened and stained as in experiment 14.

Experiment 17. — Oct. 20, 1893.

White and brown mongrel. $\frac{3}{5}$ grm. morph. sulph was injected, followed by ether.

Operation. — The right ulnar and median were resected to the extent of 8 ctm., and a segment of equal length removed from a cat's sciatic was implanted, and sutured above by a single direct catgut suture to the ulnar and median, and below to the peripheral ulnar. The wound was closed in the usual way.

Oct. 27. Wound closed ; healing by first intention.

Dec. 17. Wound entirely healed ; the dog seemed to use the foot as before the operation.

Jan. 1. No ulcers on the foot.

Examination. — Feb. 17, 1894 (117 days after implantation).

The sensation in the foot as tested by pricking with a needle seemed normal.

On exposing the nerve, a slight enlargement was seen at the lower end of the stump, and a narrow band, showing here and there faint longitudinal striation, could be traced from the central bulb to just above the central end of the peripheral ulnar, where it was lost in connective tissue.

Physiological tests :—

(1) Stimulation with induction shocks at 13 ctm. S. C., of the peripheral ulnar just below the peripheral wound, gave movements of flexion in the muscles supplied by the ulnar. This was especially plainly seen in the flexor carpi ulnaris. There was also evidence of pain as shown by reflex movements. Stimulation at the same place, after cutting the central ulnar stump 6 ctm. above the central wound and after cutting median and musculo-spiral nerves and resecting them as far as possible, gave similar results except, of course, no signs of pain.

(2) Stimulating the central ulnar at 18 ctm. S. C. above implanted segment, after severing from the center, gave marked contraction in the muscles supplied by the ulnar.

(3) Direct stimulation of muscular branch to flex. carp. ul. gave good contractions at 15 ctm. S. C.

(4) Mechanical stimulation, such as striking the nerve with a small rubber hammer at a point one inch below the peripheral wound, gave flexion of flex. carp. ul.

(5) Contraction was produced in the muscle supplied by ulnar when the nerve was cut with scissors above the implanted segment, and cutting the muscular branch below the peripheral wound caused flex. carp. ul. to contract. Unfortunately the record does not state whether the nerve was stimulated at the wrist or not.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 18. — Aug. 18, 1893.

Small white Spitz. Hypodermic injection of $\frac{2}{5}$ grm. morph. sulph. followed by ether.

Operation. — The ulnar of the right side was resected to the extent of 8 ctm., and 8 ctm. of a cat's sciatic implanted, and wound closed.

Aug. 30. Wound healed by first intention.

Examination. — Dec. 12, 1893 (120 days after operation).

The dog was prepared for an operation on the posterior extremity. $\frac{3}{5}$ grm. morph. sulph. was given ; while sterilizing the instruments the dog died from the effects of the morphia. As the apparatus for testing the nerves was not at hand, and the dog was dead some time before the dissection could be made, no reliance can be placed upon the results.

Exposing the nerves showed the peripheral end of the central ulnar to be bulbous, and the peripheral ulnar of normal appearance. Spanning between the two stumps of the ulnar could be seen four or five very fine glistening threads of the appearance of nerve fibres. Stimulation of the peripheral ulnar (thirty-five minutes after the death of the dog) excited no movement and no reflexes.

I was not able to tell whether the apparent failure in the experiment was due to the time intervening between the death of the animal and the physiological tests or not. The experiment is recorded because it shows the only incident where, after a period of more than 120 days after the implantation, regeneration through the implanted segment had not taken place. The histological examination of the nerve involved in this experiment, showed four well-formed nerve bundles in the upper part of the implanted segment, but no trace of these could be seen in the lower portion.

Experiment 19. — Jan. 23, 1894.

Brown mongrel. $\frac{2}{5}$ grm. morph. sulph. injected.

Operation. — The ulnar of the right side was exposed, and 6 ctm. removed; a segment of equal length taken from a cat's sciatic was implanted, sutured above and below with a single catgut suture, and wound closed.

Jan. 31. Two epidermal stitches had given way. The wound healed a few days later.

Examination. — June 23, 1894 (121 days after operation).

There were no trophic ulcers on the foot. The dog used the foot very well in walking up and down stairs. Exposing the nerves showed the peripheral end of the central ulnar stump to be slightly bulbous. The peripheral ulnar was normal in appearance, and the implanted segment firmly imbedded in fibrous tissue.

Physiological tests:—

(1) Stimulation with induction shocks at 15 ctm. S. C. of peripheral ulnar below implanted segment excited contractions of muscles supplied by the ulnar; and evidence of pain was shown by the reflexes, but the latter were not very marked.

(2) After severing the central ulnar stump about 6 ctm. above the central wound, and cutting the median and musculo-spiral, stimulation of the central stump produced good contraction of muscles in ulnar area at 15 ctm. S. C.

(3) Direct stimulation of the muscular branch to flex. carp. ul. and flex. profund. dig. at 15 ctm. S. C. excited contractions of these muscles, which were very clearly seen after the muscles were exposed.

(4) Stimulation of peripheral ulnar at wrist, at 2 ctm. S. C., excited no movement of the digits and no evidence of pain.

(5) Stimulation of ulnar in the middle of forearm produced feeble reflexes at 5 ctm. S. C., showing some regeneration of sensory fibres.

(6) Cutting the nerve with scissors gave distinct contractions, the nerve being cut both above central wound and below peripheral wound. Cutting the muscular branches to flex. carp. ul. and flex. profund. dig. also produced contraction, but no contraction resulted when the nerve was cut at the wrist.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 20. — Jan. 24, 1894.

Brown and white spaniel. Hypodermic injection of $\frac{2}{3}$ grm. morph. sulph.

Operation. — The left ulnar was exposed, and a segment 7 ctm. removed; a piece of equal length taken from a cat's sciatic was implanted, sutured above and below with a single direct catgut suture, and the wound closed.

Jan. 31, 1894. Wound healed by first intention.

Examination. — May 5, 1894 (121 days after operation).

Electrical stimulation with induction shocks after exposing the nerve in the arm gave the following results :—

(1) Stimulation of peripheral ulnar just below implanted segment at 12 ctm. S. C. gave good contraction of the muscles supplied by the ulnar. There was much pain as shown by reflex movements. The median and musculo-spiral were cut and resected before the tests were made.

(2) Stimulation of central ulnar just above implanted segment at 12 ctm. S. C., and after severing the central ulnar about 4 ctm. above the central wound, gave marked contraction of the flexor muscles supplied by the ulnar nerve.

(3) Stimulation of muscular branch to flex. carp. ul. at 12 ctm. S. C. gave contraction of this muscle.

(4) No contraction resulted when the ulnar was stimulated at the wrist at 5 ctm. S. C. The nerve was not stimulated at this point before the central ulnar was cut.

(5) Mechanical stimulation by cutting the nerve with scissors above the implanted segment, below the implanted segment, and cutting of muscular branches to flex. carp. ul. and flex. profund. dig., called forth marked contractions in muscles supplied by the ulnar and its branches. There was no movement of the digits when the ulnar was cut at the wrist.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 21. — Dec. 20, 1893.

Large black mongrel. $\frac{3}{5}$ grm. morph. sulph. injected, followed by ether.

Operation. — The right median nerve was exposed and resected to the extent of 5 ctm., and 5 ctm. of a cat's sciatic implanted, and sutured above and below with a single direct catgut suture, and the wound closed. The wound healed by first intention.

Examination. — May 5, 1894 (136 days after operation).

Stimulation with induction current gave following results :—

(1) Stimulation of median below implanted segment at 15 ctm. S. C. gave contraction in muscles of forearm supplied by median, and much pain as shown by well-developed reflexes. Equally marked flexion resulted upon stimulating the peripheral median after severing the central median 4 ctm. above central wound.

(2) Stimulating the central median just above the implanted segment at 15 ctm. S. C. produced well-developed movement of flexion.

(3) Direct stimulation at 12 ctm. S. C. of several of the small muscular branches given off just below the elbow, gave contraction in muscles supplied by them.

(4) Mechanical stimulation by cutting the nerve with scissors produced very marked contractions.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 22. — Nov. 25, 1893.

White bulldog. $\frac{3}{5}$ grm. morph. sulph. injected.

Operation. — The right ulnar was exposed and resected to the extent of 4 ctm., and an equal segment of cat's sciatic implanted, sutured above and below with a single direct catgut suture, and wound closed in the usual way.

Dec. 13, 1893. The epidermal wound was still open and lined by granulations. The connective tissue seemed to have healed over the implanted nerve and ends of ulnar stump.

Examination. — April 23, 1894 (149 days after operation).

The dog could use the foot apparently as well as before the operation, and the sensation in the foot seemed normal.

Tests with induced current as follows : —

(1) Stimulation of peripheral ulnar a short distance below implanted segment (after cutting and resecting median) at 18 ctm. S. C., gave good contractions of muscles supplied by ulnar, and slight reflexes ; marked reflexes when stimulated at 9 ctm. S. C. Stimulation of peripheral ulnar at 18 ctm. S. C., after severing the central ulnar about 5 ctm. above the central wound, excited marked movements of flexion.

(2) Stimulation of central ulnar (after separation from center), at 18 ctm. S. C., gave good contractions in muscles supplied by the nerve, feeble contractions when stimulated at 24 ctm. S. C.

(3) Direct stimulation of muscular branch to flex. carp. ul. at 18 ctm. S. C. gave movements of flexion.

(4) Stimulation of ulnar at wrist at 9 ctm. S. C. called forth movements of flexion in digits.

(5) Cutting the ulnar above implanted segment, below implanted segment, and cutting the branches to flex. carp. ul. and flex. profund. dig., was followed by marked contraction of the muscles supplied. Cutting the ulnar at the wrist was followed by feeble contraction of digits.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

In experiments 23 and 24 the results were almost identical with those recorded for experiment 22.

Experiment 25. — Nov. 29, 1893.

Young mastiff about one year old. $\frac{4}{5}$ grm. morph. sulph. injected, followed by ether.

Operation. — The ulnar of right arm was exposed, and a segment 6 ctm. long removed. An equal segment taken from a cat's sciatic was implanted, and sutured above and below with a single direct catgut suture.

Dec. 4. Greater portion of the wound healed by first intention ; the lower end of the wound was open and lined by granulations.

Examination. — May 30, 1894 (182 days after operation).

Wound healed. Sensation in foot normal, as far as could be determined by pricking the parts. On exposing the ulnar only a very slight enlargement was noticed on the end of the central stump.

Electrical examination with induced shocks gave the following results :—

(1) Stimulation of peripheral ulnar at 10 ctm. S. C., just below peripheral wound, caused much pain, as shown by well-developed reflexes, and called forth muscular contractions in the muscles supplied by the ulnar. Similar contractions resulted when stimulated after the central ulnar was cut, and after the median and musculo-spiral were resected.

(2) Stimulation of peripheral ulnar at wrist at 10 ctm. S. C. (a distance 7 inches below peripheral wound) caused movements of toes and quite well developed reflexes, showing regeneration of at least some of the sensory and motor fibres as far down as the foot.

(3) Stimulation of central ulnar at 15 ctm. S. C. produced movements of flexion.

(4) Direct stimulation of the muscular branches to flex. carp. ul. and flex. profund. dig. at 10 ctm. S. C. produced contraction in these muscles.

(5) Mechanical stimulation resulting from cutting the nerve with scissors excited strong muscular contractions, the nerve being cut above and below implanted segment, at the muscular branches, and at the wrist.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 26. — March 9, 1894 (secondary implantation).

Young black and brown hound. $\frac{3}{5}$ grm. morph. sulph., then ether.

Operation. — Six ctm. of the right ulnar was removed, and wound closed.

March 14. Wound healed.

April 19 (forty-one days after this operation). The ulnar was again exposed. The end of the central stump terminated in a large bulb which was imbedded in fibrous tissue. No nerve fibres were seen to extend below the bulb. The central end of the peripheral ulnar was not enlarged.

Electrical examination :—

The central bulb was very sensitive, as shown by the marked reflexes when only very weak stimuli were used. The peripheral ulnar was completely degenerated. The end of the central stump was resected to a line just above the bulb, and the peripheral ulnar vivified. The space between the two ulnar stumps amounted to about 7 ctm. A piece of equal length was taken from a cat's sciatic and implanted, and was sutured above and below to the ulnar stump with a single direct catgut suture. The connective tissue was united over the nerve with five buried sutures, and the skin with a continuous silk suture. Wound healed by first intention.

Sept. 21, 1894 (155 days after implantation) the dog was examined. Sensation in the foot was not impaired. On exposing the ulnar, a small bulb was seen at the end of central ulnar ; this was not sensitive.

Electrical examination :—

(1) Stimulating the peripheral ulnar below the implanted segment at 8 ctm. S. C. gave good contractions of muscles supplied by this nerve, and

marked evidence of pain as shown by reflexes ; this after the median had been cut and resected.

(2) Stimulation of the ulnar above the implanted segment, after severing the ulnar from the center, gave marked contractions at 12 ctm. S. C.

(3) Stimulating the ulnar in the middle of the forearm gave feeble reflexes at 8 ctm. S. C.

(4) Stimulating at wrist caused no movements and no reflexes.

Regeneration seems to have taken place through the implanted segment to a little below the middle of the forearm.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

(*b*) TUBULAR SUTURE BONE TUBE IMPLANTATION.

The experiments of others, notably those of Vanlair, had caused me to look with favor on the use of the bone tube in operations for remedying

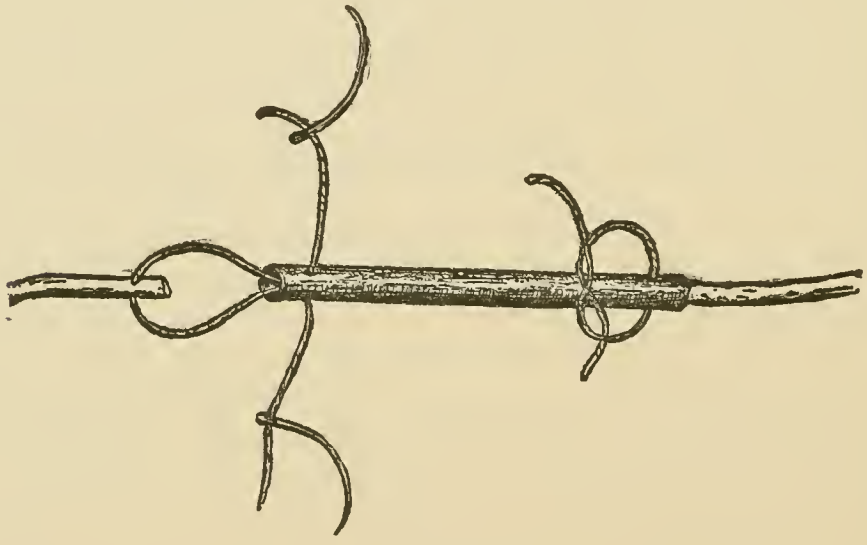

FIG. 1.

nerve defects, but the results here recorded do not fulfill this anticipation. The bone tubes used were prepared in the following manner : The ulnars of chickens and turkeys were decalcified, after cutting off the ends, in one per cent hydrochloric acid, then thoroughly washed in flowing water, placed into ether for a few hours, and finally transferred to a five per cent solution of carbolic acid, where they remained until needed. Just before using, the bone tubes were boiled for fifteen minutes in alcohol, and then placed in sterilized distilled water, from which they were implanted. After resecting the nerve to the desired extent, the central and peripheral ends of the resected nerve were inserted into the ends of the bone tube for a distance of about 1 ctm., and held in place by a single suture above and below, passed through the sides of the tube, and at the same time through the ends of the nerve inserted within its lumen. The mode of procedure is

shown in Fig. 1. The connective tissue was then united over the bone tube by means of four to six buried chromatized catgut sutures, and the edges of the epidermis brought together by silk sutures. By closing the wound in this way it was found that, while the silk sutures might break through, the deep sutures would aid the connective tissue in uniting over the bone tube and keeping it in place.

Experiment 27. — May 30, 1894.

Young mongrel. $\frac{2}{5}$ grm. morph. sulph. injected.

Operation. — The ulnar of the left side was exposed for a distance of 8 ctm., and resected to the extent of 7 ctm. The ends of the resected nerve were inserted into the ends of a bone tube about 8 ctm. long, and held in place by a catgut suture passed through the nerve and bone tube, and the wound closed.

Examination. — June 4, 1894 (five days after operation).

Epidermal wound open, connective tissue healed over bone tube, and covered with healthy granulations. On removing the granulation tissue a transparent flat band attached above and below to the resected ulnar could be recognized, presumably the implanted bone tube. Electrical stimulation of peripheral ulnar stump showed it to be degenerated.

The nerve was hardened in Flemming's solution, and stained in safranin and licht grün.

Experiment 28. — May 30, 1894.

Full-grown shepherd dog. Hypodermic injection of $\frac{3}{5}$ grm. morph. sulph.

Operation. — The left ulnar was resected 6 ctm., and the resected ends inserted into a bone tube, and retained in place by catgut sutures. Wound closed.

Examination. — June 9, 1894 (ten days after operation).

The greater portion of the wound healed by first intention. On exposing the ulnar the implanted bone tube was not recognized. The end of the central stump was slightly enlarged. The peripheral ulnar was degenerated, as shown by electrical and mechanical stimulation.

The nerve was hardened in Flemming's solution, and stained in safranin and licht grün.

The same physiological result was obtained in experiment 29. The nerve was removed, hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 30. — Dec. 15, 1893.

White and brown mongrel. $\frac{2}{5}$ grm. morph. sulph. injected, followed by ether.

Operation. — The left sciatic was exposed, and resected to the extent of 3 ctm. The central and peripheral ends of the nerve were drawn into the ends of the bone tube, and sutured in this position. Wound closed.

Jan. 1, 1894. Wound nearly healed.

Feb. 1, 1894. Dog used the foot in walking, sometimes touching the floor with the plantar surface of the foot, at other times with the dorsum of the foot. He used the foot in walking up stairs, but often did not reach far enough, and missed the step.

Examination. — Feb. 7, 1894 (fifty-four days after operation).

Electrical stimulation of central sciatic above bone tube, even with strong stimulus, produced no movement in the muscles supplied by the sciatic. The same results were obtained when the peripheral sciatic was stimulated below the bone tube, and when the internal and external popliteal branches were stimulated.

No contraction resulted from cutting the central sciatic.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 31. — Jan. 24, 1894.

White and brown spaniel. Hypodermic injection of $\frac{3}{5}$ grm. morph. sulph.

Operation. — A segment 6 ctm. long was removed from the right ulnar, the cut ends inserted within the ends of a bone tube, and sutured. Wound closed.

Examination. — May 5, 1894 (121 days after operation).

The end of the central stump was slightly enlarged.

Electrical stimulation : —

(1) Stimulation of ulnar below bone tube at 5 ctm. S. C. produced slight evidence of pain, as shown by feeble reflexes, but no movement in muscles supplied by ulnar.

(2) Stimulation above the bone tube caused no movements. There was no contraction of the muscles supplied by the ulnar when the nerve was cut with scissors central to the bone tube. It would seem that a few sensory fibres had reached the peripheral ulnar.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 32. — Dec. 13, 1893.

White bulldog. $\frac{3}{5}$ grm. morph. sulph. injected.

Operation. — The right sciatic was exposed, and 5 ctm. exsected. The cut ends of the nerve were placed into the ends of a bone tube, retained in place by catgut sutures, and wound closed.

Dec. 18. Greater portion of the wound healed by first intention.

Jan. 1894. Extensive ulcer on the dorsal surface of the right hind foot, and the toes red and swollen. In walking the dog dragged this foot, and when used for support touched the floor with the dorsal surface.

Feb. 15. No improvement in utility and appearance of the foot.

April 23. Foot not so red and swollen, ulcerating surfaces granulating, and the dog seemed to have better control of the foot than he had for the first three months after the operation.

Examination. — April 23 (121 days after operation).

The peripheral end of the central stump ends in a large bulb, which is very sensitive.

Physiological examination resulted as follows: —

(1) Stimulating sciatic below the bone tube with strong induction shocks caused no movement and no reflexes.

(2) Stimulating the sciatic above the bone tube with induction shocks at 5 ctm. S. C. produced no contraction of the muscles supplied by the sciatic. Cutting the nerve above the bone tube caused no movement of the muscles.

(3) Stimulating the external and internal popliteal with strong induction shocks gave no contraction of the foot.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 33. — Dec. 20, 1893.

Large black mongrel. $\frac{3}{5}$ grm. morph. sulph. injected.

Operation. — The right ulnar was resected to the extent of 5 ctm., the ends united with the bone tube, and wound closed.

Examination. — May 5, 1894 (130 days after operation).

Healing ulcer on the outer surface of the foot.

Stimulation of the peripheral ulnar just below the bone tube with induction shocks at 9 ctm. S. C. produced scarcely perceptible reflexes, and no flexion. Reflexes not increased with strong stimulus. Stimulation of the central ulnar above the bone tube at 5 ctm. S. C. excited no contraction in muscles supplied by ulnar. Mechanical stimulation, by cutting the nerve above and below the bone tube, excited no movement.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 34. — March 9, 1894.

Black and brown hound. Hypodermic injection of $\frac{3}{5}$ grm. morph. sulph., followed by ether.

Operation. — The left ulnar was resected to the extent of 6 ctm., and the wound closed without suturing the nerve.

April 19 (forty-one days after resecting the nerve). The ulnar was again exposed. The peripheral end of the stump presented a large and very sensitive bulb. No nerve fibres could be traced beyond this enlargement. The central end of the peripheral ulnar was not enlarged, and proved to be completely degenerated, as shown by its not responding to induction shocks. The ends of the central and peripheral stump were vivified, leaving a space of about 7 ctm. between the resected ends. These were inserted into the ends of a bone tube and sutured. The wound was then closed in the usual manner.

Examination. — Sept. 21, 1894 (155 days after second operation).

(1) Stimulation of the peripheral ulnar just below the bone tube with induction shocks at 8 ctm. S. C. gave flexion of the muscles supplied by the nerve, together with signs of pain, as shown by the reflex movements; this after the median had been resected.

(2) Stimulating central ulnar above bone tube at 12 ctm. S. C. excited contraction of the muscles supplied by ulnar.

(3) Stimulation of muscular branch to flex. carp. ul. at 8 ctm. S. C. excited contraction of this muscle.

(4) Stimulation of the ulnar at wrist gave no contraction and no reflexes.

(5) Cutting the nerve with scissors above the bone tube, below the bone tube, and cutting muscular branch to flex. carp. ul. excited contraction of muscles supplied by the nerve.

(*c*) Implantation of Catgut Bundle.

In the following series of seven experiments, threads of catgut, as suggested by Glück and Assaky, were employed to unite the ends of the resected nerve.

Experiment 35. — May 30, 1894.

Young mongrel. $\frac{3}{5}$ grm. morph. sulph.

Operation. — The ulnar of the right side was exposed, and 6 ctm. removed. In place of the suture à distance, as suggested by Assaky, the author, in this and five other experiments, employed a bundle of catgut

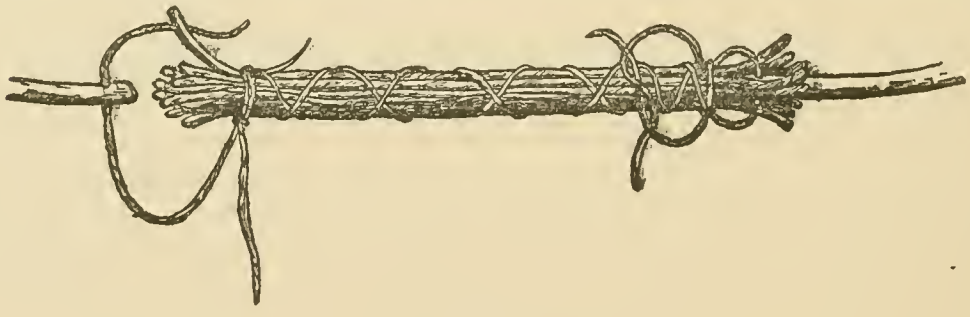

Fig. 2.

threads about 1 ctm. longer than the exsected nerve segment, and composed of eight No. 3 chromatized threads about which were wound two very fine catgut threads. The ends of the threads encircling the bundle were allowed to remain long, so that they might be employed as sutures for uniting the implanted bundle to the cut ends of the nerve. A bundle of catgut threads thus prepared and made antiseptic by boiling for fifteen minutes in alcohol and then washing in sterilized distilled water, was implanted and sutured above and below to the central and peripheral ends of the resected nerve, care being taken to have the ends of the several catgut threads composing

the implanted bundle, come up over and form a sheath or a cuff around the ends of the nerve stump. The form of the catgut bundle, and the manner in which it was united to the resected nerve ends is shown in Fig. 2. The wound was closed by stitching the subcutaneous connective tissue over the implanted catgut, and closing the dermal incision with interrupted silk sutures.

Examination. — June 4, 1894 (five days after operation).

Wound nearly healed by first intention. On opening the wound the catgut bundle was found imbedded in a mass of granulation tissue, and when isolated, was found to be quite firmly united to the central and peripheral portion of the resected nerve by means of granulation tissue.

Stimulation of the peripheral ulnar with strong induction shocks excited no movements in the muscles supplied by ulnar. No contraction was observed when the nerve was cut with scissors.

The nerve was hardened in Flemming's solution, and stained in safranin and licht grün.

The same results were obtained in experiments 36 and 37.

Experiment 38. — April 20, 1894.

Small black bulldog. $\frac{3}{5}$ grm. morph. sulph.

Operation. — The ulnar of the left side was exposed, and 5 ctm. removed. In this case the central and peripheral ends of the nerve were united by a long catgut suture, carried forward and backward three times, and at each turn the catgut thread was passed through the nerve stump, Assaky's suture à distance. The wound was then closed.

Examination. — May 30, 1894 (39 days after operation).

Wound healed. Physiological tests as follows :—

(1) Stimulation of the peripheral ulnar below the catgut threads with strong induced current gave no contraction of the muscles and no reflexes.

(2) Stimulation of the central ulnar above the implanted catgut bundle excited no contractions.

(3) Cutting the nerve with scissors excited no contraction of muscles supplied by ulnar.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 39. — Jan. 25, 1894.

Brown mongrel. $\frac{3}{5}$ grm. morph. sulph., followed by ether.

Operation. — A segment 5 ctm. in length was removed from the left ulnar, the resected ends united by suture à distance, and wound closed.

Jan. 31. Wound healing by first intention.

Examination. — June 8, 1894 (136 days, four and a half months, after operation).

Peripheral end of central stump terminated in a large bulb.

(1) Stimulation of peripheral ulnar just below catgut suture with induced current at 12 ctm. S. C. excited strong contraction of muscles supplied by

ulnar; reflexes feeble. Contraction not so marked after the central ulnar was cut.

(2) Stimulation of central ulnar just above catgut suture, after it had been divided at a point about 6 ctm. above suture, excited muscular movement at 12 ctm. S. C.

(3) Direct stimulation, at 9 ctm. S. C., of muscular branch to flex. carp. ul. excited feeble contraction of this muscle.

(4) Stimulating the peripheral ulnar in forearm caused no contraction.

(5) Stimulation at 12 ctm. S. C. of a narrow band, presumably a new-formed nerve trunk extending from central to peripheral ulnar, caused strong contraction of flex. carp. ul.

(6) Cutting the central ulnar, peripheral ulnar, and muscular branch to flex. carp. ul., with scissors, excited strong contractions.

The physiological examination leads to the conclusion that the new nerve fibres had reached the upper portion of peripheral ulnar and the muscular branch to flex. carp ul., but as yet had not grown any distance down the peripheral stump.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 40. — Dec. 20, 1893.

Large black mongrel. Hypodermic injection of $\frac{3}{5}$ grm. morph. sulph., followed by ether.

Operation. — The ulnar of the left side was resected 5 ctm., and a bundle composed of eight No. 3 catgut threads implanted, sutured to the resected ends of the ulnar, and wound closed.

Examination. — May 5, 1894 (136 days after operation).

(1) Stimulating with strong induction current of peripheral ulnar below the catgut bundle excited no reflexes and no contraction of muscles supplied by ulnar.

(2) Stimulating central ulnar after severing the nerve 5 ctm. above the implanted catgut bundle caused no contraction.

(3) Cutting the central ulnar, peripheral ulnar, and muscular branch to flex. carp. ul., excited no contractions.

Conclusion. — No return of function to sensory or motor fibres in the peripheral ulnar.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 41. — Dec. 2, 1893.

Shepherd dog. Hypodermic injection of $\frac{3}{5}$ grm. morph. sulph., followed by ether.

Operation. — The left ulnar was resected to the extent of 4 ctm., and a catgut bundle implanted and sutured above and below to the resected ulnar. Wound closed.

Examination. — May 30, 1894 (152 days after implantation).

(1) Electrical stimulation with induced current at 12 ctm. S. C. of peripheral ulnar just below peripheral wound excited muscular movements and reflexes.

(2) Stimulating the central ulnar, after cutting the nerve about 5 ctm. above the central wound and after resecting the median, excited strong contractions of muscles in ulnar region, at 24 ctm. S. C.

(3) Direct stimulation of muscular branch to flex. carp. ul. at 12 ctm. S. C., excited contraction of the muscle.

(4) Very feeble movements of the digits were observed when the peripheral ulnar was stimulated at the wrist with strong induction shocks.

(5) Cutting the central ulnar, peripheral ulnar, and muscular branch to flex. carp. ul. with scissors, excited strong contraction of the muscles supplied.

Conclusion. — Nearly complete regeneration of peripheral portion of resected nerve.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

(*d*) Létiévant's Flap Operation.

In the following seven operations, the value of a nerve flap, as suggested many years ago by Létiévant, was tried as a method for uniting the widely separated ends of a divided nerve.

Experiments 42 and 43. — June 2, 1894.

Black spaniel. $\frac{3}{5}$ grm. morph. sulph.

Operation. — (Exp. 42, left ulnar; 43, right ulnar.) The ulnar nerves of the left and right sides were exposed, and in each case resected to the extent of 5 ctm. The distance separating the central and peripheral stumps of the ulnar was bridged by a nerve flap made from the central stump. To make this flap the following steps were taken: a thin sharp knife was passed through the substance of the central stump about ½ ctm. from its end, the cutting edge pointing central-ward. Without removing the knife, the incision was continued up the nerve trunk for a distance equaling in length the space separating the ends of the resected nerve, care being taken to bisect the nerve, as nearly as possible, into equal parts. Before removing the knife, the upper end of one of the halves was severed from the undivided central ulnar; the flap so formed was then turned down, and its free end stitched by means of a catgut suture to the central end of the peripheral ulnar. The wound was then closed in the usual manner.

Examination. — June 7, 1894 (five days after operation).

Electrical stimulation of the central ulnar, of flap, and of peripheral ulnar excited no contraction of muscles supplied by nerves operated upon.

The nerves were hardened in Flemming's solution, and stained in safranin and licht grün.

Experiments 44 and 45. — June 2, 1894.

White and brown mongrel. $\frac{3}{5}$ grm. morph. sulph.

Operation. — No. 44, right ulnar ; 45, left ulnar.

After ten days there was degeneration of peripheral ulnar.

Experiment 46. — April 20, 1894.

Large yellow and black mongrel. $\frac{3}{5}$ grm. morph. sulph., followed by ether.

Operation. — The right ulnar was exposed, and 6 ctm. cut out. A flap was made from the central stump and sutured to the peripheral ulnar, and wound closed.

Examination. — June 23, 1894 (64 days later).

Wound completely healed, and the dog did not seem to suffer any inconvenience in the use of the extremity. On exposing the ulnar the peripheral end of the central stump was slightly enlarged.

(1) Electrical stimulation of peripheral ulnar at 5 ctm. S. C., just below its junction with the flap, excited no contraction and no reflexes.

(2) Stimulating in the region of the flap, after freeing it by dissection, caused pain, as shown by reflex movements, but no contraction.

(3) Stimulating the central ulnar with a strong current gave no contractions.

Conclusion. — There was a down-growth of sensory fibres for a short distance below the end of the central stump, but not into the peripheral ulnar.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 47. — March 6, 1894.

Full-grown greyhound. $\frac{3}{5}$ grm. morph. sulph. injected, followed by ether.

Operation. — A segment 5 ctm. long was removed from the right ulnar, and a nerve flap made from the central stump was turned down and stitched to the peripheral ulnar. Wound closed.

Examination. — July 31, 1894 (147 days after).

Electrical stimulation of the exposed ulnar, below the peripheral flap, with a strong induced current excited no contraction of muscles in ulnar area, and gave no evidence of pain as would be shown by reflexes.

Cutting the ulnar at various points gave the same negative results. The physiological examination showed no regeneration.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

Experiment 48. — March 6, 1894.

Full-grown greyhound. $\frac{3}{5}$ grm. morph. sulph. injected, followed by ether.

Operation. — Removed 5 ctm. of the left ulnar. The free ends of two nerve flaps, one made from the central stump about 3 ctm. long, the other

from the peripheral stump and of equal length, were brought together, sutured by means of a direct catgut suture, and wound closed.

Examination. — July 31, 1894 (147 days after).

Electrical stimulation of the ulnar below flaps excited no contraction and no reflexes. The same negative results were obtained when stimulating above the flaps, and when stimulating the muscular branch to flex. carp. ul.

The nerve was hardened in Müller's fluid, and stained in anilin blue and safranin.

(*e*) Nerve Grafting.

Experiment 49. — Nov. 25, 1893.

Full-grown white bulldog. Hypodermic injection of $\frac{3}{5}$ grm. morph. sulph.

Operation. — The ulnar and median nerves of the left arm were exposed, 4 ctm. removed from the median, and the central end of peripheral median stump was stitched by means of three catgut sutures to the accompanying ulnar. Before stitching, a small area of the trunk of the ulnar was denuded of its connective tissue sheath. The central median stump was allowed to end free in the wound. The wound was closed with catgut and silk sutures.

Examination. — April 23, 1894 (149 days after).

After removing the skin and subcutaneous connective tissue, the peripheral median stump was found to be firmly united to the accompanying ulnar. The central median stump terminated in a large bulb.

Electrical stimulation of the peripheral median showed it to be degenerated ; no contraction of muscles supplied, and no reflexes were observed.

(*f*) Létiévant's and Tillmanns' Operation, that is, Cross-Suturing the Long Ends and Grafting the Short Ends of Two Parallel Nerves Cut at a Different Level.

Experiment 50. — Nov. 29, 1893.

Young mastiff about one year old. $\frac{4}{5}$ grm. morph. sulph., followed by ether.

Operation. — The ulnar and median were exposed. From the two nerves were exsected segments 4 ctm. long. The portions removed were taken from different levels of the two nerves. As will be seen from Fig. 3, the ulnar was divided much higher up than the median, and as the segments removed from the two nerves were of about the same length, the peripheral stump of the ulnar was longer than that of the median. In cases where, as in the present experiment, an incision passes obliquely over two nerves having a parallel course, and where so much nerve substance is lost that the respective ends cannot be brought together, Tillmanns recommends a "cross-suturing" of the longer ends, and a grafting of the shorter stumps

to the accompanying nerve trunk. Tillmanns' suggestion was made use of in this operation. The longer central stump of the median was sutured to the longer peripheral stump of the ulnar. The short central stump of the ulnar was grafted (stitched) to the central median, and the short peripheral

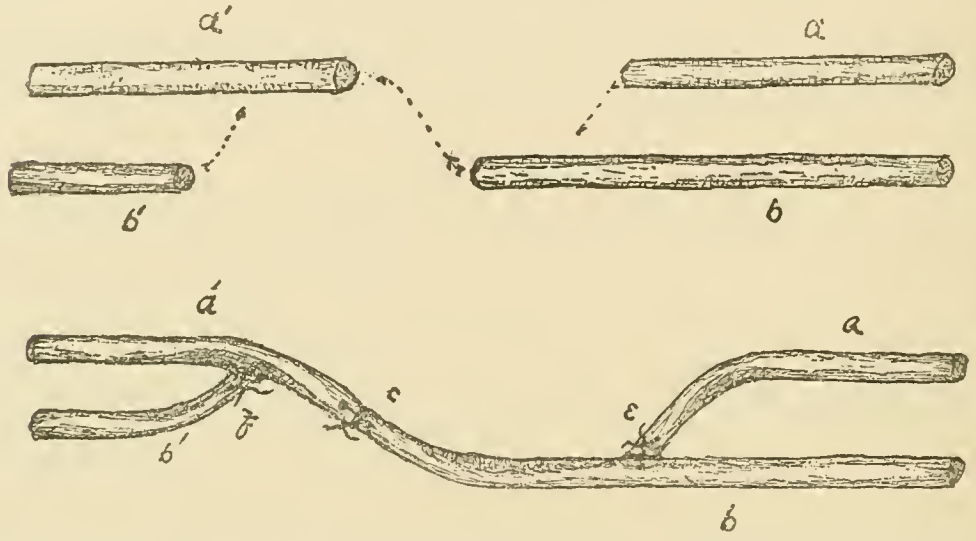

Fig. 3. —*a*, central ulnar stump ; *a'*, peripheral uluar stump : *b*, central median stump ; *b'*, peripheral median stump ; *c*, cross-suture of central median and peripheral ulnar segment ; *e*, grafting of peripheral end of central ulnar segment to median ; *f*, grafting of central end of peripheral median stump to ulnar.

stump of the median to the peripheral ulnar as shown in Fig. 3. The connective tissue was carefully stitched over the nerves by means of numerous buried catgut sutures, and the skin wound closed with silk sutures.

Dec. 14, 1893. Nearly the entire wound healed by first intention.

Examination. — May 30, 1894 (182 days after operation).

On laying bare the nerves in the arm, the following conditions were met with : —

The central median stump was found united to the peripheral segment of the ulnar. The central ulnar stump was fixed to the median by means of connective tissue, and on careful dissection was found to end in a bulb. The peripheral median was attached to the peripheral ulnar.

Physiological examination gave the following results :—

(1) Electrical stimulation by induction shocks at 15 ctm. S. C. of peripheral ulnar below its junction with median, excited strong contraction in muscles supplied by ulnar, and much pain, as shown by the well-developed reflexes.

(2) Stimulating the central median above junction with the ulnar, with induction shocks at 15 ctm. S. C., gave strong contraction of flex. carp. ul. and flex. prof. dig., as could easily be seen on observing these muscles after their exposure.

(3) Direct stimulation of the muscular branches to the above muscles excited contraction.

(4) Stimulating the peripheral ulnar at wrist excited movements of the digits and reflex movements, showing a return of motor and sensory function of ulnar branches in the foot.

(5) The central ulnar stump, which was, as will be remembered, grafted to the median, was cut 8 ctm. above the point of grafting, and then stimulated by induction shocks at 5 ctm. S. C., but no movement could be observed in the muscles supplied by the median or ulnar.

(6) The peripheral stump of the median, which had been grafted to the ulnar, was found to be degenerated.

(7) Mechanical stimulation by cutting the median above its junction with ulnar, by cutting the peripheral ulnar, and by cutting the muscular branches, excited contraction of flex. carp. ul. and flex. prof. dig.

(8) No contraction resulted after cutting the central ulnar and peripheral median stump.

Conclusion. — Complete regeneration of peripheral ulnar through fibres of the central median stump. The engrafted stump of median was degenerated.

The nerves were hardened in Müller's fluid, and stained in anilin blue and safranin.

Part III. — Histological.

Methods. — Of the great number of methods employed by the various writers who have made a study of degeneration and regeneration of divided peripheral nerves, very few bring out with clearness the changes undergone by the axis cylinder. The fate of the myelin can be quite easily traced if the degenerating nerve is hardened in a fluid having osmic acid as one of its constituents. The structural changes undergone by the nerve nuclei can be readily followed in preparations hardened in Flemming's or Hermann's solution, and stained in safranin. After much experimenting with the various methods used for staining the axis cylinder in peripheral nerves, the author came to the conclusion that, as far as his experience goes, the best results were to be obtained with Stroebe's anilin blue and safranin stain. This method, slightly modified, was used in preparing the great majority of the histological preparations. In each experiment the entire peripheral stump of the operated nerve, the implanted piece, and about one inch of the central stump, were removed and hardened from three to four weeks in Müller's fluid, at a temperature of forty degrees C.; then washed in

flowing water for about thirty minutes, and placed into ninety-five per cent alcohol for three or four days.

(To keep the nerve fibres straight during the process of hardening the following device was made use of: One end of each of two small glass rods, the one about eight, the other about three inches long, was pushed through a cork of a size to fit a six-inch test tube ; the other ends of the two rods were bent in the form of a hook. After removing a nerve segment from a dog its central end was tied to the short hook, its distal end to the long one, and the long rod was moved up or down in the cork until the nerve attained the proper degree of extension. The hardening was then carried on in a test tube.)

After remaining in ninety-five per cent alcohol the required time, each nerve was divided into pieces varying in length from one-half to one inch. Two consecutive segments, one of which was used for longitudinal, the other for transverse sections, were placed in properly labeled test tubes, in which they were carried through absolute alcohol, toluol, soft and hard paraffin, and were then imbedded in a mixture composed of four parts of hard paraffin and one of spermaceti.

Sections varying in thickness from five to ten mikrons were cut and fixed to a cover glass by a method described by the author in a former paper. The method consists in floating the paraffin sections on distilled water, then carefully warming the water until they flatten out, which they usually do very readily. A thin layer of "albumin fixative" is now spread on a cover glass ; this is then inserted under the sections floating on the water, one or more of which are drawn up on the cover glass, a small sable-hair brush being used for this purpose. When this has been done, the cover slip with the sections on it is drawn out of the water and set aside, best in a warm oven at forty degrees C. for several hours. Nearly all the albumin fixative is washed away, so it does not interfere with the staining, yet enough seems to remain on the cover to cause the section to adhere more firmly than when water is used, as recommended by Gaskell, or when fifty to seventy per cent alcohol is employed, as recommended by Gaule.

Cutting in paraffin admits of thinner sections than when

celloidin is used, as Stroebe recommends, and the technic of staining is simpler. The cover glass can be handled much more easily than thin celloidin sections. Then, too, one can often fix ten to fifteen sections to one cover, so there is a great saving of time. The paraffin is removed in the usual way, and then the cover with the sections on it is carried through "the alcohols" into distilled water.

The technic of Stroebe's anilin blue-safranin method is as follows:—

(1) Sections are stained from one to five hours in a saturated aqueous solution of anilin blue (Grübler's wasserlösliches Anilinblau).

(2) They are then rinsed in distilled water.

(3) Decolorized in an "alkali alcohol wash," which is prepared by dissolving 1 grm. of caustic potash in 100 c.c. of absolute alcohol, and filtering. Just before using thirty to forty drops of this solution are mixed with 30 c.c. of absolute alcohol. On placing the sections into this wash they lose their blue color, and assume a reddish-brown tinge; at the same time reddish-brown waves are given off from the preparation. The bleaching is continued until the reddish-brown waves cease to form, which usually takes about one to two minutes. If many sections are to be bleached it will be found necessary to renew the alkali wash.

(4) The sections are now transferred to distilled water, in which the blue color again returns, of a much lighter hue, however. The sections remain in the water about ten minutes.

(5) Counter-stain in a saturated aqueous solution of safranin for about one-half hour.

(6) Rinse in distilled water.

(7) Wash and dehydrate quickly in ninety-five per cent, then in absolute alcohol.

(8) Clear in oil of bergamot, and place the sections into xylol, from which they are mounted in Canada balsam. The xylol leaves a clean surface on the cover after evaporating, and is for this reason to be used after oil of bergamot.

The axis cylinder will be stained deeply blue, the myelin reddish-yellow, or of an orange tinge, and the nerve nuclei, and all

other nuclei, of a safranin color, the protoplasm a faint red. In case the safranin stain is extracted too much the blue stain will predominate in the section. Yellow elastic tissue will sometimes stain very much like an axis cylinder, but the definite outline of the elastic fibres, and their relation to other structures, will make it possible to obviate any misinterpretation. In a number of experiments, especially those in which the earlier degenerative changes of myelin and the behavior of the nerve nuclei were made the subject of study, the nerves were hardened in Flemming's solution (extension being maintained in the manner described for Müller's fluid-hardened nerves), imbedded in paraffin, sectioned and fixed to the cover glass, and doubly stained with safranin and licht grün, after Benda's method.

In making teased preparations it was found quite helpful to cut sections twenty to thirty mikrons thick, remove the paraffin, stain in anilin blue and safranin, or safranin and licht grün, and then tease these stained sections in oil of bergamot, and mount in balsam. Sections, especially such as contain much fibrous tissue, can thus be easily teased, and the nerve fibres are, as a rule, not so liable to be broken up as when teased in the usual way.

(*a*) *Degeneration and Regeneration after Implantation of a Nerve Segment.*

The degenerative changes which befall the implanted segment do not differ materially from this process as observed in peripheral nerves after severance from its center, except that the changes take place more rapidly.

By the end of the second day the myelin and axis cylinders of the nerves of the implanted segment are found to be broken up into larger and smaller fragments, and there is already an increase of the protoplasm within the sheath of Schwann. At this stage the segments of myelin vary largely in size and shape : some are long, and entirely fill the sheath of Schwann in that portion of the degenerating fibres in which they are found ; others are in the form of small, round, or oval balls,

surrounded by the proliferated protoplasm ; between these extremes one finds every gradation. The segments are usually surrounded by a layer of myelin varying in thickness, and showing a decided difference in the extent to which it is stained with osmic acid. The protoplasm has a homogeneous appearance, staining faintly red in safranin. I am unable to state, as a result of the preparations examined, what is the cause of the fragmentation of the myelin and axis cylinder. In the sections at my disposal the degenerative changes were too far advanced to make any observation on this point. It does not, however, seem probable that the fragments of myelin bear any relation to the so-called segments of Lantermann or Schmidt. Their irregular shape and size would exclude such a possibility. The nerve nuclei are found in larger number than in normal fibres, where, as is well known, a single internodal nucleus is present. As a rule they occupy a position in the middle of the fibre between two segments of myelin. It is somewhat difficult to estimate their number, as the nodes of Ranvier cannot be clearly made out at this stage. To some extent, at least, the proliferation of the nuclei would seem to result from karyokinetic division of preëxisting internodal nuclei ; now and then one is found in the early stages of cell division. I was not fortunate enough, even after looking through a large number of sections, to find a nucleus in the monaster or diaster stage. The nucleus shown in the middle one of the five fibres reproduced in Pl. XXXIV, Fig. 1, which represents a portion of a longitudinal section of the implanted segment two days after the operation, presents a structure differing from that of the majority of the nuclei seen. The chromatic filaments (chromosoma) are clearly discerned, and the nucleoli are wanting, although the nuclear membrane is still present; while the resting nuclei present a very delicate nuclear network, and usually two, three, or four prominent nucleoli. Now and then one finds a nucleus of hour-glass or dumb-bell shape, which leads one to think that some of the nuclei fragment (amitotic division), and in this way contribute to the proliferation of the nuclei seen. A great number of polynuclear white-blood cells are found in the lymph spaces between the connective

tissue bundles of the epineurium, also between the junction of the central ulnar stump and the central end of the implanted segment, and between the peripheral ulnar and the peripheral end of the implanted nerve. From the central and peripheral wound they may be traced for a short distance between the nerve fibres of the implanted segment, in less number between the nerve fibres of the peripheral and central ulnar in the neighborhood of the wounds. I was not able to find any polynuclear leucocytes within the sheath of Schwann of the nerve fibres in the transplanted segment, nor within the ends of the nerve fibres of the central and peripheral stump of the ulnar. The nerve fibres of the peripheral and central portion of the ulnar are apparently of normal structure, the axis cylinder is not interrupted, and the myelin presents a normal appearance. That the fibres of the peripheral end possessed conductivity is shown by the fact that the muscles supplied by the ulnar contracted when the nerve was cut about 3 ctm. below the peripheral wound. The "traumatic degeneration," as described by Bünger, Stroebe, and others, extends only a short distance along the fibres of the central and peripheral ulnar, and is a process differing essentially from the "secondary degeneration," which involves the entire peripheral portion of the severed nerve. In the immediate neighborhood of the trauma the myelin is broken up into fragments, and in some of the fibres the sheath of Schwann is filled with a granular *débris*, staining a gray-black in osmic acid. The axis cylinder is, as a rule. unbroken, and can be seen imbedded in the myelin.

During the third day segmentation of the myelin in the implanted fibres continues, and the fibres begin to show a very irregular shape, many of them showing nodular enlargements, in which are found one or several large fragments of myelin; these alternate with segments of the nerve of much smaller diameter, composed largely of proliferated protoplasm in which may be imbedded smaller drops or granules of myelin. A chemical change has taken place in many of the fragments of myelin, evinced by the way in which they stain with osmic acid and safranin. In a normal nerve, hardened in osmic acid (Flemming's solution) and stained with safranin, the myelin

assumes a black color, and is not touched by the safranin. The same fact is observed during the early stages of degeneration (myelin black and the protoplasm of a reddish tinge); as degeneration proceeds, one sees that the myelin begins to be stained also by the safranin, and now assumes a color which is a mixture of the black and red stain. This change takes place more rapidly near the nuclei. There is no marked increase of the nuclei of the sheath of Schwann over that observed at the end of the second day, although one finds now and then one in mitosis, as is seen in Pl. XXXIV, Fig. 2 *b*. The nuclei are less numerous in a degenerating fibre of the implanted segment than would be observed in degenerating fibres of the peripheral end at a corresponding stage. Polynuclear leucocytes are found in large numbers in the endoneural connective tissue between the nerve fibres at this stage (see Pl. XXXIV, Fig. 2), especially so in the outer portion of the implanted nerve trunk. The fact that they are first observed in the epineurium, at a little later stage in the lymph space between this sheath and the nerve fibres, finally between the nerve fibres near the epineurium, leads to the conviction that they wander in through the connective tissue sheaths surrounding the implanted nerves. At this stage the endoneural connective tissue cells are proliferating by means of mitotic division, as is shown in Pl. XXXIV, Fig. 2 *d*. The peripheral portion of the ulnar is up to this time of normal histological structure, and stimulation with induction shocks excites contraction of the muscles supplied.

During the fourth and fifth days the fragmentation and absorption of the myelin in the implanted fibres goes on quite actively, yet not with the same rapidity in the different fibres nor in different portions of the same fibre. The myelin would seem to be absorbed more rapidly than the axis cylinders. In longitudinal sections and teased preparations made from an implanted nerve taken from a dog five days after the operation, and stained in anilin blue, irregular segments of the axis cylinder, stained deep blue with the dye, are seen in nearly every fibre. Some of these fragments are long, now and then slightly twisted (see Pl. XXXIV, Fig. 3 *a*), often ending in

bulbous enlargements. These larger segments are often found free in the proliferated protoplasm filling the sheath. About some of them are found layers of fine granules concentrically arranged, stained like the fragments of the axis cylinder ; they may show the manner in which the axis cylinder segments are resolved. The shorter "axes segments" are as a rule surrounded by a thin layer of myelin of a yellowish color. In the above preparation polynuclear leucocytes are found in large numbers between the nerve fibres, and between the ends of the implanted segments and the stump of the ulnar. The spindle-shaped cells ("specific nerve granulation cells"), described by Glück as developing between the cut ends of a nerve, and as uniting the central and peripheral axis cylinders, were in no case observed. During the fourth and fifth days degeneration begins in the peripheral end of the divided nerve. The myelin and axis cylinders begin to be broken into fragments, and some of the nuclei of the sheath of Schwann are in process of karyokinetic cell division. An inevitable result of the fragmentation of the axis cylinders is the loss of the conductivity of the fibres in the peripheral nerve, as shown by the fact that they do not carry impulses when stimulated with induction shocks or mechanically. The permeation of the polynuclear leucocytes, as observed in the implanted segment, was not observed in the degenerating peripheral ulnar.

During the sixth, seventh, and eighth days, fragmentation and absorption of the myelin and axis cylinders of the fibres in the implanted segment goes on so rapidly that by the end of the ninth day it becomes very difficult to recognize the implanted nerve. At this time the nearly collapsed sheaths of Schwann take a green stain (tissue hardened in Flemming's solution and stained in safranin and licht grün), and contain a protoplasm in which are found a few granules faintly tinged with the safranin (see Pl. XXXIV, Fig. 4). At rare intervals the observer meets larger or smaller balls of myelin stained black with the osmic acid. One such fibre is shown in the figure ; at either side of the myelin fragments the tubular sheath becomes rapidly smaller, and resembles in structure the other fibres reproduced. The nuclei of the sheath of Schwann

are not numerous ; they, as a rule, occupy a central position in the collapsed sheath, and are surrounded by the protoplasm. Between the degenerated fibres are found spindle-shaped connective tissue cells and polynuclear leucocytes in larger number than the figure would lead one to infer ; and not unfrequently capillaries or small arterioles filled with red and white blood cells are met with. Pl. XXXIV, Fig. 5, represents a portion of a typical nerve fibre taken from the peripheral ulnar nine days after the operation for implantation. A comparison of this with Pl. XXXIV, Fig. 4 (portion of implanted segment of the same dog), shows that in the degenerating fibres of the peripheral ulnar segment, the absorption of the myelin has not advanced nearly so far, while the proliferation of the nuclei is more marked than in the fibres of the implanted segment. That this proliferation of the nuclei takes place after the manner of a typical mitosis has been shown by Torre, Bünger, Stroebe, and myself in a former article, and I am, at this time, able to corroborate the observation already recorded. The peripheral end of the central ulnar stump degenerates to the extent of about ¾ ctm. The degeneration is essentially the same as observed in the peripheral ulnar. Between the central end of the implanted segment and the peripheral end of the central ulnar stump is seen a layer of embryonic connective tissue, composed largely of branched and spindle-shaped connective tissue cells and polynuclear leucocytes enclosed in the meshes of a very loose fibrous reticulum. A narrow layer, of similar structure, unites the peripheral end of the implanted segment and the peripheral ulnar.

From the ninth to the twenty-first day very little structural change worthy of mention is observed in the fibres of the implanted segment. In long. sect. of this tissue, removed twenty-one days after the operation, hardened in Müller's fluid, and stained in anilin blue and safranin, the collapsed sheaths are not well made out. In teased preparations (see Pl. XXXIV, Fig. 7) they appear as narrow tubes, often compressed and containing a small amount of protoplasm and here and there a nucleus. Between the fibres are found connective tissue elements and leucocytes. In the peripheral ulnar of the above

experiment, the myelin and axis cylinders had been entirely absorbed in the great majority of the fibres. The old sheaths are filled with a continuous band of protoplasm, imbedded in which are found the nuclei, with their long diameter parallel to the longitudinal direction of the fibre (see Pl. XXXIV, Fig. 8 *a*). Even at this stage, however, remnants of the medullary sheath are found in some of the fibres; and, in these, it is sometimes possible to find small fragments of the old axis cylinder (see Pl. XXXIV, Fig. 8 *d*), which take a deep blue color when stained in anilin blue. The longitudinal striation of the protoplasm as described by Bünger, in which he recognizes the anlage for the new axis cylinders, was at no time observed. The protoplasm has a homogeneous appearance and stains faintly blue in sections colored in anilin blue and safranin, in case the safranin is again thoroughly washed out of the specimen; or may have a faint reddish color in preparations overstained in the safranin. By far the most interesting observations to be made on the operated nerve, examined twenty-one days after implantation, are to be found in the longitudinal sections or teased preparations through the junction of the central ulnar and the implanted piece. In this region the process of regeneration is already established. As the regeneration of the peripheral portion of a divided nerve, and especially the regeneration of the axis cylinders, has been a subject on which observations have been largely at variance, I may be allowed to state some of the opinions expressed. We have, in the first place, the views upheld by Schiff, Laveran, and Remak; that during the degeneration of the peripheral nerve, the axis cylinder is not affected, but retains its structure and forms the axes of the new fibres. That this is not the case has been clearly shown a great number of times. All axis cylinder stains show, that, during degeneration of the nerve fibres, its continuity is broken, and the resulting fragments are always absorbed. Neumann and Eichhorst contend that during degeneration of the peripheral end, the medullary sheath and axis cylinder undergo a chemical change of such a nature that, structurally and chemically, it is not possible to differentiate between the two; "the process of regeneration

consists in a differentiation of this common substance into a new axis cylinder and a new myelin" (see Howell and Huber, from whom this quotation is taken). Neumann believes that the new axis cylinder is developed in short pieces, one for each internodal segment. This segmental anlage is, according to Neumann, seen in the peripheral end of the central portion of the injured nerve, in the wound (in his experiment, the continuity of the axis cylinders and myelin was interrupted by means of a ligature tied very firmly around the nerve and then removed), and in the central end of the peripheral stump, extending from here to the periphery. Bünger states that the new axis cylinders are developed from the proliferated protoplasm and nuclei of the degenerating fibre. By the end of the second week after the injury, the protoplasm becomes longitudinally striated, and is differentiated into a band-like structure, the new axis cylinder. The development takes place in short segments, which later unite to form the band, Stroebe and Notthaft, in two very careful researches, have critically reviewed Bünger's results, and find no evidence in support of his statements. Among those who believe that the new axis cylinder is developed from some one of the cellular elements in the peripheral portion of the injured nerve, may be mentioned Hjelt and Wolberg, who trace its origin from the cells of the connective tissue surrounding the nerve fibres ; and Einsiedel, Korybutt-Daskiewics, Beneke, and Leegard, who maintain that the proliferated nuclei of the sheath of Schwann are the anlage for the new axis cylinders. The present conception of the structure and development of the axis cylinder, according to which it is regarded as a long process extending uninterruptedly from the nerve cells to its place of distribution, either in a motor or sensory ending, makes it difficult to accept any one of the above views. In support of this assertion, we may finally give the observation of a goodly number of investigators, who maintain that the new axis cylinder is formed during regeneration after the manner of its development in the embryo, that is, in the form of a bud or a sprout, which grows from the ganglion cell (or its severed process, the axis cylinder), toward the periphery.

This conception of the regeneration of the new axes was first defended by Ranvier, and has since been held by Vanlair, Assaky, Howell and Huber, Stroebe, Notthaft, and Willard. After this brief summary, of the views held concerning the formation of the axis cylinder during the process of regeneration, I will now turn to the results obtained in my experiments. In longitudinal sections and teased preparations through the junction of the central end of the ulnar and the implanted segment, made from two nerves removed twenty-one days after the implantation (Exps. 14 and 15), the following observations are to be made:—

At this time it is quite easy to determine the extent to which the central end has degenerated, as, in the area marking the upper limit of the degenerated part of the fibres, the myelin is found in larger or smaller fragments, and does not present the normal appearance seen in the same fibre a short distance nearer the center. In these fragments of myelin one now and then finds remnants of the old axis cylinder taking the characteristic stain. In many of these fibres nothing is seen of the new axis cylinder in that part containing the fragments of myelin, or just below, while it is clearly made out in the normal portion of the fibres just above. In others, the new axis cylinder can be traced for a short distance into the portion of the fibre containing the fragments of myelin; here it often has a somewhat tortuous course, and ends free in the proliferated protoplasm. The fragments of myelin are not disposed around the axis cylinder in the form of a sheath, but are usually to the side of it. In still other fibres, the axis cylinder can be traced through the segment of the nerve, showing the broken up myelin, into the more distal portion of the degenerated central fibre, which presents structurally the appearance of a completely degenerated nerve, that is, a collapsed sheath of Schwann containing a homogeneous nucleated protoplasm. One such fibre is represented in Pl. XXXIV, Fig. 6. The central end of the fibre points toward the top of the page. In this part the axis cylinder is surrounded by the medullary sheath; just below are seen a number of fragments of myelin; between them the somewhat wavy axis cylinder is winding its

way towards the periphery into the completely degenerated part of the fibre, ending free in the protoplasm ; ten nuclei of the sheath are seen in this short segment. In the layer of connective tissue found between the central ulnar and implanted segment are to be seen a few very fine threads stained deeply blue, surrounded by a thin layer of a pale blue color. They are found between the connective tissue fibrillae and cellular elements constituting the layer just mentioned. I was often able to trace them through several fields of the microscope when studied with a $\frac{1}{12}$ inch oil immersion, but found no example where I could observe their connection with one of the fibres of the central end. In order to see these fine new axis cylinders clearly, for that is what they undoubtedly are, it is necessary to cut very thin sections, 5 to 7 mikrons in thickness ; and, as their course is always somewhat irregular, one can easily see that a section, embracing a portion of the central nerve fibre and a young axis cylinder in connection with it, as it winds its way through the developing connective tissue for a distance of about ½ ctm., would be exceedingly rare. Stroebe was able to see such a connection. We find on p. 208 of his article the following statement : "Nicht selten treten jedoch auch Beziehungen der schmalen Axencylinder in der Druckstelle zu jenen mit kolbiger Endanschwellung versehenen Axencylindern des centralen Stumpfes auf, welche bei den früheren Stadien beschrieben wurden (see Pl. VII, Fig. 1, 2, and 3. bei K. A. of his article). Man sieht die junge Faser sich vom alten Axencylinder centralwärts von dessen terminaler Verdickung dort, wo der Axencylinder, abgesehen von geringer Ausschwellung, wieder normal ist, abzweigen, dann in der alten Scheide weiter peripherwärts ziehen und so zwischen die Zellzüge der Compressionsstelle eintreten." Stroebe used rabbits in his experiments, compressing the "large ear-nerve" with an instrument constructed by him. The observations above recorded were made on nerves removed from the rabbit seven days after compression, hardened in Müller's fluid, and stained in anilin blue safranin. Even after the most careful search in longitudinal and cross-sections, and teased preparations made from the implanted portion (Exps.

14 and 15) just below the central wound, no axis cylinders were seen ; one meets only the degenerated fibres shown in Pl. XXXIV, Fig. 7. This, together with the fact that there are no elements in the connective tissue (if our conception of the structure of the axis cylinder is correct) from which the few axis cylinders there found might have been developed, would, I think, justify the assumption that they are buds from the central axis cylinders ; although, in my preparations, I am not able to make out a connection between the axis cylinder of the central fibre and the axes found in the connective tissue just below.

At thirty-nine days after the implantation new axis cylinders are found in the implanted segment, in the peripheral wound, and in the central end of the peripheral ulnar. When sections of the central wound are compared with the ones made from the same region of a nerve removed twenty-one days after implantation, a very much larger number of axis cylinders is evident in the connective tissue uniting the central ulnar and the implanted segment. Here some are found arranged in small bundles, others are disposed singly. The bundles and single fibres are often seen deviating from a course parallel to the axis of the nerve. They can be traced above into the central stump, and below into the implanted segment. The deeply stained axis cylinders are surrounded by a narrow border tinged faintly blue. Long, rod-shaped nuclei with rounded or beveled ends are observed in the pale blue layer, closely applied to the new axis cylinders. In cross-sections of the implanted segment, about 1 ctm. below the central wound, a small portion of one of which is reproduced in Pl. XXXIV, Fig. 9, the degenerated fibres of the implanted nerve are seen as small, oval, or round tubes, the wall of which is formed by the old sheath of Schwann, containing a homogeneous protoplasm which scarcely stains. In a few of the collapsed fibres a nucleus is observed. This section presents an appearance differing from a cross-section of a degenerated nerve trunk at a similar stage, in the large amount of connective tissue found between the fibres. This connective tissue, which, as far as my observation goes, always develops around and between the elements of the

implanted segment, separates the degenerated nerve fibres into smaller and larger bundles, around which it is often arranged in the form of a sheath. In such a cross-section the relation of the newly formed axis cylinder to old sheaths is well seen. The deeply stained dots, representing cut ends of the axes, are now and then found within an old sheath, as shown in Pl. XXXIV, Fig. 9 *c*. Often a very delicate sheath, found within the old one, surrounds them. Others are seen by the sides of the degenerated sheaths, as shown in *b* of this figure. In longitudinal sections and teased preparations of the peripheral portion of the implanted segment, relatively few axis cylinders are found; in Pl. XXXV, Fig. 10 *b*, two are represented, among a larger number of degenerated implanted fibres (*a*). In the connective tissue uniting the peripheral end of the implanted segment to the peripheral ulnar still fewer axis cylinders are observed. In longitudinal sections of this part of the nerve, several fields are often passed before one is met with. Their course is much more irregular than that presented by the axes seen in the connective tissue of the central wound. Preparations made from the peripheral ulnar about 1 ctm. below the peripheral wound, show the great majority of the fibres in complete degeneration. Only at very rare intervals is a characteristically stained axis cylinder found. The appearance presented in one of my teased preparations from this locality is given in Fig. 11. The entire peripheral ulnar from a point about 1 ctm. below the peripheral wound to the wrist, as also the muscular branches to flex. carp. ulnaris and flex. prof. dig., were divided into small pieces, which were used, some for cross, others for longitudinal sections; but in none of these did I meet with an appearance which might have been interpreted as representing an axis cylinder. It is very interesting to recall that in this experiment (No. 16) stimulation of the peripheral ulnar just below the peripheral wound, with strong induction shocks, excited no contractions of the muscles supplied by this nerve; however, impulses would seem to have been carried along the few regenerated nerve fibres (some of which, no doubt, were sensory) found in the upper end of the peripheral ulnar and the peripheral

wound, as the record of the experiment shows that feeble reflexes were observed. I may briefly summarize the above results, and the interpretation given them in the following statement: many of the down-growing axis cylinders from the central nerve fibres have reached the connective tissue layer between the central stump and the implanted segment; of this number a very small per cent have reached the upper end of the peripheral ulnar, some others the peripheral wound and peripheral portion of the implanted segment, and a larger number the middle and central end of the implanted nerve.

The regeneration of the peripheral ulnar is far advanced by the end of the fourth month. A review of the results obtained at the physiological examination in Exps. 17, 19, and 20, show a return of function to the motor fibres going to flex. carp. ul., and also that the sensory fibres have the power of conducting impulses, at least down to the middle of the forearm (Exp. 19). Microscopical examination corroborates these conclusions. It is at this time very easy in longitudinal sections to trace the axis cylinders of the fibres in the central end through the connective tissue into the upper end of the implanted segment. Usually the old axis cylinder ends in a slight enlargement, from which, or from the axis cylinder just above it, a smaller thread, stained deeply in anilin blue, the new axis cylinder, surrounded by a narrow sheath of myelin, can be traced. Now and then an appearance shown in Pl. XXXV, Fig. 12, is seen. In such cases the central axis cylinders would seem to have divided into several branches, these growing for a longer or shorter distance toward the periphery within the old sheath of Schwann. In the fibre reproduced in the figure, only one of the new axis cylinders becomes the axis of a nerve fibre; the others are seen to end in small bulbous enlargements. Similar observations have been made by Ranvier, and by Howell and Huber (Fig. 60, and not so well represented in Fig. 62), and very clearly shown in Figs. 8, 9, and 10 of Pl. VIII, illustrating Stroebe's article. The course of the nerve fibres through the central wound (the connective tissue between the central stump and implanted segment) I have tried to represent in Pl. XXXV,

Fig. 13, in which is reproduced a part of a longitudinal section through this region from a nerve removed 117 days after implantation. Some of the small bundles, it will be seen, have a definite direction, parallel to the long axis of the nerve; others have a wavy or twisted course (*c–c*), and one small bundle, *d*, is seen in cross-section. A study of many sections from this region gives me the conviction that the new nerve fibres are winding their way through the spaces in the connective tissue.

In the implanted portion the fibres are arranged in bundles, separated by fibrous connective tissue, which varies in amount in different parts of the implanted nerve. As seen in cross-section, the bundles show a closer proximity in the axial part than they do toward the borders. Here one meets with small bundles, separated entirely from the other nerve fibres, and some distance from them. Each small bundle is surrounded by a distinct layer of connective tissue forming a perineural sheath. The distance separating the bundles is much greater in the peripheral part of the implanted nerve than nearer the central wound, and the bundles are not so numerous. (See Pl. XXXV, Figs. 17 and 18.) The former shows a portion of a cross-section of the central, the latter of the peripheral end of an implanted segment 157 days after operation. In longitudinal sections of the implanted piece a large number of the nerve fibres have a direction parallel to the axis of the nerve, but are often slightly wavy; some of the small bundles, especially such as occupy a position near the borders of the implanted nerve, pass out of it, and are lost in the surrounding connective tissue.

Very interesting observations are to be made on longitudinal sections of the peripheral wound in the nerves of the experiments above referred to (about 120 days after implantation). Pl. XXXV, Fig. 14, presents an appearance commonly met with. The course of the axis cylinders is here very much more irregular than in any other part of the regenerated nerve. The new axes are often seen deflected to one side or the other, and it is not unusual to see them turned back, and growing toward the center for a short distance. In sections, owing to

their tortuous course, only short segments of axis cylinders are met with. Many are lost in the connective tissue surrounding the nerve trunk, and do not reach the peripheral ulnar. With one of the nerves a small portion of the striped muscle tissue, a part of one of the forearm flexors, on which the ulnar rests just above the elbow, was removed. In sections through this, several small bundles composed of six to eight nerve fibres, having a very tortuous course, were seen in the endomysium between the muscle fibres. I attribute the conditions here met with to the fact that before the down-growing axis cylinders of the central end reach the peripheral wound, the developing connective tissue between the lower end of the implanted portion and the peripheral ulnar has already become quite firmly organized, and offers much greater resistance to the down-growing axes than does the connective tissue of the central wound which, at the time when the new fibres are first seen, is more embryonic in its character. For it will be remembered that in the experiments, where twenty-one days intervened between the implantation and the removal of the nerve, a few newly formed axis cylinders were found in the connective tissue layer between the central stump and implanted nerve. Beyond this no new fibres were observed, and by the end of the thirty-ninth day after the operation the greater number of the new axis cylinders had passed the central wound, and could be traced a shorter or longer distance into the implanted nerve, while only very few are seen in the lower wound and central end of the peripheral ulnar.

Still another interesting fact is shown in teased preparations and longitudinal sections of the peripheral ulnar, and of the muscular branches to the flex. carp. ul. in the above experiments. The preparations show that the regenerated fibres are found in larger numbers in the central end of the peripheral stump and the muscular branches, the proportion becoming much smaller in sections made from the peripheral part of the nerves. In two of the experiments (Nos. 19 and 20) no axis cylinders are seen 2 ctm. below the middle of the forearm; while in Exp. 17 very few were present in the ulnar at the wrist. Teased preparations show that some of the regenerated

fibres are to be seen within the old sheaths, while others are found between them. Now and then the axis cylinder will be seen to end abruptly in the protoplasm of one of the degenerated old fibres. In Pl. XXXV, Fig. 15 *A* (in which portions of three fibres from the muscular branch to the flex. carp. ul. are represented), the axis cylinders end in a bulbous enlargement at *c*, beyond which the old sheath (*s*) could be traced through several fields of the microscope. Above the bulbous enlargement the axis cylinder is enclosed within a narrow layer of myelin (*n m*), and surrounding this is seen a new sheath of Schwann. *B* of the same diagram presents essentially the same condition, except that the end of the axis cylinder is not enlarged, but has for a short distance a wavy course. (I would crave indulgence for the awkward way in which the fibres in Pl. XXXV, Fig. 15, are placed. It will be noticed that the peripheral end of one [*A*] points toward the right, while that of the other [*B*] points toward the left. The sketch was made from teased preparations, and the error was not noticed until the diagram was completed.) This same appearance was met with in other parts of the peripheral ulnar, as is shown in Pl. XXXV, Fig. 16 *E* (from a teased preparation of the peripheral ulnar at the wrist 117 days after operation. Exp. 17). In this fibre the new axis cylinder is not surrounded by a medullary sheath. In the nerves from this experiment nearly all the fibres of the peripheral ulnar at the wrist present the appearance of a completely degenerated nerve (see Pl. XXXV, Fig. 16 *A*). In a few of the degenerated fibres unabsorbed fragments of myelin are still present (Pl. XXXV, Fig. 16 *B*). One such fibre containing a new axis cylinder is shown in *D* of the same figure.

The appearance presented in specimens made from nerves removed 149 to 182 days after implantation (Exps. 21 to 25) differ from the ones above described only in the extent of regeneration. I may briefly state that the longer the interval between the implantation of the nerve and the time of its removal, the more complete is the return of function, and the more regenerated fibres are to be seen in the peripheral end of the injured nerve. In all of the last-named experiments elec-

trical stimulation of the ulnar at the wrist excited distinct movements of the digits; and microscopical examination of the nerve in this region revealed the presence of regenerated fibres. Pl. XXXV, Fig. 19, shows the extent of regeneration at the wrist 182 days after implantation. It will be seen that many of the fibres possess axis cylinders, and some of the fibres (*a*) present the structural appearance of a normal nerve.

Only one experiment of secondary implantation of a nerve was made. The right ulnar of a dog was resected 6 ctm., and without suturing the ends of the nerve the wound was closed. After a period of forty-one days the nerve was again exposed. At this time the end of the central ulnar segment was much enlarged, and seemed very sensitive. The ulnar was redivided just above the bulb, and after vivifying the central end of the peripheral ulnar a segment of cat's sciatic was implanted. Sections of the portion removed from the peripheral stump of ulnar showed that the nerve fibres had undergone complete degeneration.

Sections of the bulb (Stumpfneurome, amputations or Durchschneidungsneurome) removed from the central ulnar stump present the appearance so often described for such structures. Kölliker states that the bulbs must be looked upon as expressing the regenerative energy of the nerve, an hypothesis which, no doubt, expresses correctly the existing conditions. Structurally this bulb consisted largely of small groups of new nerve fibres very complexly interwoven between bundles of fibrous tissue. Such neuromes would seem to develop from the degenerated part of the central stump and the connective tissue surrounding its end. Above the bulb the fibres of the ulnar presented a normal appearance.

The dog was killed 155 days after the second operation. The results of the physiological and histological examination of the nerve involved in this experiment are so similar to the ones obtained in experiments of primary implantation of about 120 days' duration, which have already been recorded, that a detailed description of them at this stage would only be a repetition of observations already given.

I have purposely avoided making any mention of the develop-

ment of the medullary sheaths about the newly formed axis cylinders, as the anilin-blue safranin method (used for staining sections made from all nerves where the process of regeneration was made the subject of study, from 21 to 182 days after implantation) is not a suitable stain for making out the origin of this sheath. In the author's sections prepared after this method the medullary sheath is not differentiated until a time when it has obtained some degree of prominence. Stroebe recognizes in the delicate pale blue sheath, which surrounds the more deeply stained axis cylinders, a structure already referred to several times, the beginning of the layer of myelin. He states that in sections stained after Weigert's haematoxylin method "the young fibres are stained deeply black ; they possess, therefore, at their first appearance a medullary sheath, the pale blue layer in anilin-blue preparations ; accordingly, the medullary sheath is an attribute of the young fibres from the time of their first appearance." The diagrams illustrating Willard's articles show that the medullary layer must be developed at a very early stage ; as in his preparations, stained with Weigert's haematoxylin, the nerve fibres were stained deeply black at a time when he was first able to recognize them. Bünger states that "the myelin is formed as a narrow continuous sheath, which stains black in osmic acid, and secondarily as a thicker layer which develops in segments, and at a later stage fuses with the former (an die erste anschliesst und mit derselben verschmilzt)."

If the narrow continuous sheath, which assumes a pale blue color in the anilin-blue safranin stain, is to be regarded as the anlage for the medullary layer, I think that we must admit that in its first development, it must have a slightly different chemical structure than it does at a later stage; for when it is more fully developed, in preparations stained by the above method, it has a yellowish or an orange color, even after much of the safranin has been washed out of the sections, as may be seen from Stroebe's and my own diagrams. The virtue of the anilin-blue safranin method, is to be found in its clear axis cylinder differentiation, and was for this reason used. The time at my disposal did not admit of my making duplicate

preparations fixed in osmic acid, or for the imbedding of portions of nerves in celloidin, so that Weigert's method might be used.

In each experiment alternate longitudinal and cross-sections of the portion of the nerve involved were made, so that I might, if possible, come to some definite conclusions concerning the mode of development of the axis cylinders. On some of the sections of nerves hardened in Müller's fluid and imbedded in paraffin, the Weigert method was tried, but with no results. I was, therefore, compelled to forego a closer study of the development of the medullary sheath, and confine my attention to regeneration of the axis cylinder, a structure of far greater importance.

In closing this part of the discussion, I may, however, be permitted to quote the following from Howell and Huber's article: —

"It is quite easy to find in the peripheral trunk newly regenerated fibres, showing a delicate continuous layer of myelin; but to demonstrate how this myelin is deposited is more difficult; to ascertain this one must obtain his specimens from just that portion of the nerve in which the process is actively going on. Figs. 36, 37, 39, 40, and 41 give an idea of how the myelin first appears. As shown in these figures, it appears first as irregular deposits in the protoplasm of the embryonic fibres, and usually first in the neighborhood of the nuclei. Delicate prolongations of the myelin are often seen running from one small mass of myelin to another, and eventually these latter become connected together forming a varicose tube, shown in various stages in Figs. 36–51. There would seem to be no doubt that it is first formed as disconnected drops. These may afterwards become united into slender processes to form a bead-like string, which sooner or later elongates to an even tube, or the drops may first elongate to form cylindrical segments, which eventually unite to form continuous delicate tubes of myelin." I possess at the present time no facts which would prevent my accepting the above statements.

The following brief conclusions, based on a review of the

experiments dealing with resection and subsequent implantation of a nerve segment, and a study of the microscopical appearances presented, may, for the sake of clearness, be summarized as follows:—

I. After primary implantation of a segment of a nerve trunk between the resected ends of a peripheral nerve, the implanted portion, the entire peripheral stump, and about ¾ ctm. of the central stump degenerate.

II. The myelin and axis cylinders of the implanted nerves are absorbed within the first ten days after the operation. The absorption takes place more slowly in the peripheral degenerating portion of the nerve, and in the degenerated part of the central stump.

III. The degenerated fibres of the implanted segment and injured nerve present essentially the same structural appearance, collapsed sheaths containing a nucleated band of protoplasm. The nuclei are, however, less numerous in the degenerated fibres of the implanted part than in the degenerated fibres of the peripheral and central stump of the injured nerve.

IV. Regeneration begins in the central stump, and proceeds centrifugally. It consists in a down-growth of the axis cylinders of the nerve fibres of the central end, through the central wound, the implanted segment, the peripheral wound, and the peripheral portion of the injured nerve.

V. By the end of the twenty-first day, some of the down-growing axes have reached the central wound.

VI. By the end of the thirty-seventh day, the greater proportion of the down-growing axes have passed the central wound; some few have passed through the implanted segment and the peripheral wound, and have reached the upper end of the peripheral part of the nerve. There is some return of irritability to this part of the nerve as shown by the presence of reflex movement on stimulation with strong induction shocks.

VII. By the end of the 120th day, regeneration extends to a short distance below the middle of the forearm, and to the muscular branches of the flex. carp. ul. and flex. prof. dig. (in experiments involving the ulnar). Many of the new axis cylinders

in the peripheral ulnar are surrounded by a medullary sheath, and present the structural appearance of a normal nerve.

VIII. After the 136th day in primary implantation between the resected ends of a median, and the 149th day after the same operation in the ulnar, the down-growing axis cylinders have reached the peripheral part of the injured nerve, as shown by physiological and histological examination.

IX. The down-growing axis cylinders are found in the old sheaths of Schwann in that portion of the fibres of the central stump which underwent degeneration, within and between the old sheaths in the implanted segment, and bear a similar relation to the old sheaths in the degenerated peripheral part of the nerve.

(*b*) *Microscopical Appearances in Experiments of Tubular Suture.*

A study of longitudinal and cross-sections through the region of the implanted bone tube in Exps. 27, 28, 29, with periods of five, ten, and twenty days after uniting the ends of a resected nerve with a bone drain, show that absorption of the bone must take place very rapidly, as scarcely any portion of it is found by the end of the tenth day, and no trace of it at the end of the twentieth day. Even in the experiment of five days' duration, the wall of the bone tube was so altered structurally, that it bore very slight resemblance to decalcified bone. In its place is seen a network of coarse trabeculae, very irregular in shape and size, stained very deeply red, and presenting a homogeneous appearance in preparations hardened in Flemming's solution, and stained in safranin and licht grün. The meshes of this network are filled with polynuclear white blood cells and connective tissue cells. (The bone drain used in the above experiment was made from the ulnar of a chicken, the shaft of which has only a thin layer of compact bone.) In the lumen of the implanted bone tube, in the experiment of five days' duration, is found a very loose connective tissue, rich in spindle-shaped and branched connective tissue cells, and only a few connective tissue fibrillae, irregularly interwoven, are

seen. In the other two experiments, the connective tissue between the ends of the resected nerve presents a dense structure, not so dense, however, as the connective tissue surrounding the implanted bone tube. However, my experiments were not numerous enough to admit of any full description as to the nature of the absorption of the implanted bone.

In the above three experiments, the entire peripheral portion of the nerve operated upon was found in process of degeneration, the extent of which was proportional to the time intervening between the resection of the nerve and death of the animal. The peripheral end of the central segment was degenerated to the extent of 1 ctm., while central-ward it presents a normal appearance. In the experiment of twenty days' duration, regeneration had begun in the peripheral end of the central stump. Longitudinal sections through the central wound in this experiment, stained in anilin blue and safranin, simulate very closely the appearances presented by a section through the central wound twenty-one days after the implantation of a nerve segment, which has been previously described and diagrammed in Pl. XXXIV, Fig. 6. In the undegenerated part of the fibres of the central end, the axis cylinder presents a normal appearance; it often ends distally in a small bulbous enlargement, from which or from the axis cylinders just above, can be traced, for a shorter or longer distance, fine threads stained deeply with the anilin blue (the new axis cylinders) into the degenerated portion of the central fibre. Some few of the axis cylinders of the central end show the branching diagrammed in Pl. XXXV, Fig. 12. In the connective tissue below the central stump no axis cylinders were met with.

In the experiment of fifty-four days' duration (No. 30) the connective tissue between the outer and inner ham-string muscles, which surrounded and united the central and peripheral ends of the resected sciatic, was removed, and to the eye no trace of the bone tube was visible. In longitudinal section of the peripheral end of the central stump of the sciatic and the connective tissue just below, the following observations are to be made: The sciatic is seen to end in a large bulb nearly a ctm. in diameter, and is surrounded by a dense con-

nective tissue layer. From one side of this bulb there extends into the connective tissue below the bulb, a small bundle of nerve fibres about 1 mm. in thickness, composed largely of new axis cylinders, some of which are surrounded by a thin layer of myelin. For a distance of about ½ ctm. the fibres constituting the above bundle have nearly a straight course, and are parallel to a line uniting the central and peripheral parts of the resected nerve. Just below this point the nerve bundle would seem to split up, brush-like, into larger and smaller groups of fibres; these, for the greater part, now have a very irregular course. In cross and longitudinal sections of the cicatricial tissue 1 to 2 ctm. below the central bulb only scattered nerve fibres and small bundles of fibres are met with.

In the connective tissue just above the central end of the peripheral portion of the resected nerve, I was not able to find any axis cylinders. Sections made from different parts of the external and internal popliteal show these to be completely degenerated; only the collapsed sheath of Schwann containing a nucleated band of protoplasm, with here and there a fragment of unabsorbed myelin, were seen.

In Exp. 32, of 130 days' duration, where the sciatic was resected 5 ctm. and a tubular suture made, there was no return of functional activity to the peripheral end, as tested with electrical and mechanical stimulation. Histological examination, however, revealed the presence of new axis cylinders in the connective tissue between the central and peripheral parts of the sciatic, to a point just above the peripheral wound. From the lower end of the bulb, in which the central stump terminated, bundles of small nerve fibres, some naked, others possessing thin medullary sheaths, could be traced into the connective tissue below the bulb. At first these bundles have a regular, straight, or slightly wavy course. This regular arrangement is lost about 1 ctm. from the end of the central sciatic, and below this point the connective tissue contains small bundles of nerve fibres running in every direction, the nerve fibres becoming less numerous as the peripheral wound is reached, where they are entirely wanting. The popliteal branches were completely degenerated.

In Exps. 31 and 33 (resection and tubular suture of the ulnar), of 121 and 136 days' duration, there was a slight return of functional activity to the peripheral end, as stimulation of the ulnar just below the peripheral wound excited reflexes, but no muscular contractions. Histological examination of these nerves shows new nerve fibres in the upper end of the peripheral stump, a larger number in the connective tissue between the resected ends of the nerve, where their number is inversely proportional to the distance below the lower end of the central nerve trunk. The peripheral ulnar, from a point 3 ctm. below the lower wound to the wrist, was divided into short pieces. Alternating segments were used for longitudinal and cross-sections. In none of the sections made were any axis cylinders found.

To determine whether the bone tube might be employed as a secondary operation, in cases where there was loss of nerve substance, a right ulnar of a dog was resected to the extent of 6 ctm., and the wound closed (Exp. 34). Forty-one days later the nerve was again exposed, and, after vivifying the ends, a tubular bone-drain suture made. Longitudinal sections of the segment removed from the central part of the peripheral stump, only showed degenerated fibres in the form of collapsed sheaths containing a small amount of nucleated protoplasm. Presumably the entire peripheral part of the nerve presented the same structure at the time of the second operation. The central end of the nerve was seen to end in a large bulb. The microscopical appearances presented by this nerve 155 days after the second operation may be thus briefly stated: A second bulb had formed on the end of the central stump, beyond which a large number of small bundles of nerve fibres were found in the connective tissue just below the bulb. Cross-section of the cicatricial tissue just above the central end of the peripheral part of the resected nerve shows that a relatively small proportion of the nerve fibres found higher up had reached this region, as only a small per cent of these is met with in such sections. Regeneration extends to about the middle of the forearm, and to the muscular branch of the flex. carp. ul., although only a very small number of axis cylinders

are found in this part of the nerve. In the ulnar, below the middle of the forearm, no regenerated nerve fibres were found.

The experiments of tubular suture, although not as successful in the results obtained as are the ones reported by others, notably Vanlair, seem to me to demonstrate some interesting facts. In the first place, they offer a more striking proof of the down-growth of the axes of the central end during the process of regeneration, than do experiments of ordinary nerve suture or even implantation of a nerve segment. Attention has already been drawn to this fact while reviewing the results obtained by Vanlair in Part I of this paper. In Exps. 30 and 32 new nerve fibres were found in the connective tissue between the central and peripheral part of the resected nerve, in larger number and more regularly arranged the nearer the central stump the observations were made. The peripheral part of the resected nerve of the above experiments was completely degenerated, and could therefore in no way contribute to the regeneration. And if the axis cylinder is a process of a cell of epiblastic origin, a theory which I think is now generally accepted, an independent formation of axis cylinders in the connective tissue is excluded. The experiments further show that a return of functional activity in any part of the peripheral segment of a divided nerve is concomitant with a histologically demonstrable presence of axis cylinders at that level in the peripheral nerve, and that irritability is first present near the wound and slowly extends centrifugally.

The bone tube would play only a secondary rôle in regeneration after the tubular suture. In its place there is found at the end of ten to fifteen days a loose connective tissue, which offers less resistance to the down-growing axis cylinders than does the fibrous tissue surrounding the implanted bone drain. The growing axes follow this path of lesser resistance, and some few reach the peripheral end of the nerve. An explanation as to why the new nerve fibres have a more regular arrangement in the fibrous tissue near the central wound than they show in the fibrous tissue nearer the peripheral part of the resected nerve, may be found in the fact that, at a time when the axes are beginning to grow toward the periphery,

the less developed connective tissue just below the central stump offers less resistance to them than does the same tissue at a later stage, it having attained a denser and more highly organized structure.

(*c*) *Suture à Distance and Implantation of a Catgut Bundle.*

Nearly all experimenters and operators who have studied the regeneration of a peripheral nerve after resection or after loss of substance to the extent that an ordinary suture cannot be made, recognize the necessity of establishing between the divided ends of the nerve a path along which the regenerated fibres may grow. As has been previously stated, Glück implanted strands of Danish leather, strips of muscle and tendon, bone tubes, catgut bundles, *etc.*, thinking that the implanted substance might guide the new nerve fibres, and cause them to grow in the direction hoped for. Glück met with failure in all of the above experiments. That regeneration may be attained after the use of a tubular suture, is shown by the recorded experiments of Vanlair, Bünger, and myself. The same may be said of the results obtained by Assaky after suture à distance with chromatized catgut. In the six experiments reported by him, in which this method was employed, regeneration of the peripheral end occurred in each case. In the seven experiments of suture à distance made by myself, the physiological examination (for details see Part II) showed a regeneration of the peripheral part of the divided nerve, in two out of three experiments observed for a period of more than 120 days after the operation. That the catgut threads play only a secondary part in the regeneration is shown by a microscopical examination of the nerves involved in Exps. 35, 36, and 37, of five, ten, and twenty days' duration. In sections made through the region of the catgut bundle, it will be seen that the implanted threads begin to be absorbed before the end of the fifth day, and are almost entirely absorbed by the end of the tenth day. The connective tissue about the implanted threads is seen in active proliferation in the immediate neighborhood of the bundle, and between its component threads

are seen large numbers of polynuclear leucocytes, branched and spindle-shaped connective tissue cells found in a loose fibrous tissue framework. By the twentieth day no trace of the implanted catgut is to be seen. I must, however, describe a curious appearance met with in a section of the connective tissue uniting the central and peripheral part of the resected nerve of this experiment. When such sections are examined with a low power, it will be seen that in certain parts the fibrous tissue is loosely interwoven, is rich in connective tissue and polynuclear white blood cells, and contains here and there small groups of fat cells. The portions of the section having this structure are band-like in shape. Such areas are from 1 to 2 mm. broad, and in section cut in line with the implanted catgut bundles, they now and then extend from one end to the other, in a section 1½ ctm. long. Others may have only one half or one quarter that length. Such areas have quite straight borders ; two, three, or four may be found in a longitudinal section. They are separated one from the other by a narrow band of much firmer fibrous tissue. In sections made at right angles to the implanted segment, six to eight round or oval patches of the same loose fibrous tissue, surrounded by layers of a denser connective tissue, are met with. I am inclined to think that the looser connective tissue supplants the implanted and absorbed catgut threads, while the denser tissue was developed between the threads, its formation beginning before the catgut was absorbed.

In the above three experiments, the fibres of the peripheral part of the resected nerve are found in process of degeneration. In the experiments of five and ten days' duration, the peripheral portion of the nerve fibres of the central end are found degenerated for a distance of about ½ ctm.; and in the experiment of twenty days' duration, signs of regeneration are present. In longitudinal sections of this part of the nerve, new axis cylinders are found in the degenerated part of the central fibres, and some few in the connective tissue surrounding the resected end of the central stump. The appearance presented is like that already described for sections made from this region in nerves removed about twenty days after opera-

tion in the implantation of a nerve segment, or the performance of a bone-drain suture. The down-growing axis cylinders of the central end would seem to make much slower progress in cases where the suture à distance is made, than in those where the resected ends are bridged with a segment of a nerve trunk. In Exp. 16, where a nerve segment 7 ctm. long was implanted, newly formed nerve fibres were found in the central end of the peripheral portion of the resected ulnar, and through the whole length of the implanted segment thirty-nine days after the implantation. In Exp. 38, of like duration, a segment 5 ctm. in length was removed from an ulnar and the resected ends united by a suture à distance ; in this case regeneration of the peripheral part of the ulnar was not accomplished. Small bundles of axis cylinders were found in the connective tissue about 4 ctm. below the central wound, but in cicatricial tissue surrounding the central end of the peripheral stump none were found. In longitudinal sections through the peripheral end of the central stump and the connective tissue just below, bundles of small nerve fibres, the majority of which consist only of an axis cylinder surrounded by the pale blue sheath seen in anilin blue and safranin stained preparations, are seen to extend from the undegenerated part of the central stump into the connective tissue. Here they at first have quite a regular course which, however, must soon be lost, as in sections made of the tissue 1 to 2 ctm. nearer the periphery, only short, twisted, and bent segments of the small nerve bundles, surrounded by fibrous tissue, are met with. Below this point the bundles become less numerous, and, if anything, more twisted, until, as already stated, they cannot be found. The nerve fibres found in the cicatricial tissue uniting the central and peripheral stumps in this experiment, have a much more irregular course than do the newly formed fibres seen in longitudinal sections of the implanted segment in Exp. 16, which would, I think, seem to indicate that in the former experiment the budding axis cylinders met with much greater resistance in their down-growth toward the peripheral portion of the resected nerve, than do developing axes which grow through a degenerated implanted nerve segment.

Cicatricial tissue between the resected ends of the ulnar (Exp. 38) cannot be differentiated into columns of a loosely interwoven tissue, surrounded by layers of a denser fibrous tissue, as was described for preparations made from Exp. 37, twenty days after implanting the catgut threads. It presents a structure of about the same density throughout. I am unable to state, whether the new axis cylinders which begin to grow out from the central end about twenty days after suture à distance (at a time when tracts of a looser structure are to be found in the fibrous tissue uniting the resected ends), follow these paths or not.

In the peripheral portion of the ulnar in Exp. 38, only degenerated nerve fibres, with collapsed sheaths containing a nucleated band of protoplasm, were found.

In Exp. 39 (of 135 days' duration), regeneration of the ulnar extends to about the middle of the forearm and to the muscular branch of the flex. carp. ul., as in this portion of the nerve newly formed nerve fibres are to be seen in and between the old sheaths of the degenerated fibres. Structurally, many of the nerve fibres consist only of an axis cylinder; in others a thin layer of myelin is recognized. In the peripheral ulnar from a point a little below the middle of the forearm, only degenerated fibres are found. In Exp. 41 (of 152 days' duration), sections made from any part of the peripheral ulnar show regenerated fibres. In both of these experiments, in the cicatricial tissue uniting the resected ends, bundles of nerve fibres, which have their origin in the central stump, and which can be traced from the connective tissue into the peripheral stump, are found. In the connective tissue the bundles of nerve fibres have a very tortuous course, as may be seen from Pl. XXXV, Figs. 20 and 21, made from sections of the tissue supplanting and surrounding the catgut threads in Exp. 41. Pl. XXXV, Fig. 20, represents a part of a longitudinal section cut from tissue taken from about 1 ctm. below the central wound. It will be seen that while some of the nerve bundles (*a*) have a direction which is nearly parallel to a line uniting the central and peripheral ends of the resected nerve, others (*d*) have, at least for a short distance, a course which is

at right angles to it. Many of the bundles (*c*) are cut transversely or very obliquely, and some are wavy or of a corkscrew shape. This is even more apparent in cross-sections of this tissue. Pl. XXXV, Fig. 21, represents a portion of such a section. In this some of the bundles of nerve fibres (*c*) are seen in longitudinal section. A comparison of this figure with Pl. XXXV, Figs. 17 and 18, which show portion of a cross-section of an implanted nerve segment, and the bundles of new nerve fibres which have grown through it, 152 days after its implantation, is of interest. In Pl. XXXV, Figs. 17 and 18, the bundles of nerve fibres are separated by a relatively small amount of fibrous tissue, and all the bundles are seen in cross-section, which could only be the case, if they had a course which was nearly straight, while in Pl. XXXV, Fig. 21, the fibrous tissue predominates, and the very tortuous course of the small bundles is evident from the fact that they are met not only in cross but also in oblique and longitudinal sections.

In Exp. 40 no return of functional activity was obtained. The central ulnar stump terminated in a large bulb, from which bundles of young nerve fibres are given off. They can be traced only for a short distance into the cicatricial tissue below the bulb. In the connective tissue, just above the central end of the peripheral stump, a remnant of the implanted catgut bundle, about 1½ ctm. long, was found. In cross-section, the eight threads implanted, are very clearly visible. They appear somewhat broken up, and have been permeated by polynuclear leucocytes and connective tissue cells. Why only a portion of the implanted catgut bundle was absorbed, and not all of it, I am not able to explain. It may be that the unabsorbed threads proved an obstacle to the down-growing axis cylinders which could not be overcome, and this may have prevented their reaching the peripheral ulnar, a supposition, which, if correct, would go to show that the down-growing axes reach the peripheral portion of the resected nerve through the looser fibrous tissue supplanting the absorbed catgut threads.

(*d*) *Létiévant's Flap Operation.*

Létiévant's flap operation was performed seven times (Exps. 42–48). Physiological examination of the nerves involved showed the peripheral segment of the resected nerve to be degenerated in every case. Microscopical examination results as follows: In the experiments of five (Nos. 42 and 43) and ten days' (Nos. 44 and 45) duration, the nerve fibres of the flap made from the central stump, as also the fibres of the peripheral part of the resected ulnar, are found to be in process of degeneration. In longitudinal and cross-sections of these portions of the nerves involved in the above experiments, only fibres presenting a fragmented myelin, and containing an hypertrophied protoplasm in which are to be found an increased number of the nuclei of the sheath of Schwann, are met with. The rapidity of the breaking up and absorption of the myelin is about the same in the nerve fibres found in the flap as for the fibres found in the peripheral part of the resected nerve, differing in this respect from an implanted segment, the fibres of which undergo degeneration much more speedily than do the fibres of the peripheral part of the nerve to which the implanted segment is sutured. Why this should be so is somewhat difficult to explain. The only explanation which I should care to suggest is to be found in the organic union between the flap and the peripheral end of the part of the nerve from which it was made, through which the passage of lymph and blood currents may be maintained. The more favorable nutrition thus afforded may to some extent retard the degeneration. Between the degenerated fibres of the flap are found a large number of polynuclear leucocytes. Between the peripheral end of the flap and the distal ulnar segment the presence of wandering cells and embryonic connective tissue cells, of branched or spindle shape, is to be noted.

It will be remembered that in making the flap a thin knife was pushed through the nerve trunk about ½ ctm. from its end, and without withdrawing the blade the nerve trunk was divided into two parts to the extent desired. In that part of the central stump from which the flap was cut many fibres

having a beaded appearance are to be seen. The extent of myelin fragmentation varies, however, in the different fibres. Now and then the medullary sheath is broken up, while the continuity of the axis cylinder would not seem to be interrupted. On this point, however, I can only speak tentatively, as in safranin and licht grün stained sections the axis cylinder is not very clearly differentiated. The larger number of the degenerated fibres in the central stump is found in the immediate neighborhood of the path traversed by the knife in forming the flap. The degeneration would, therefore, seem to be the result of the traumatism incurred while bisecting the nerve trunk.

In the experiment of sixty-four days' duration (No. 46) the central stump is slightly enlarged at that point in the central segment where, after bisecting the nerve preparatory to making the flap, the upper half of the end to be turned down as the flap was severed from the central end. In longitudinal sections through this region of the central segment a large nerve bunble, the fibres of which are very irregularly arranged, is observed. This bundle is given off from the peripheral end of the enlargement above referred to. It no doubt represents the half of that part of the bisected central stump which was not cut while making the flap. Many of the nerve fibres making up this bundle do not differ structurally from fibres seen in section of any peripheral nerve, while others have only a very narrow medullary layer, and still others are devoid of this sheath. I assume that the fibres presenting the appearance of fully developed medullary nerves did not undergo degeneration, while such as show only a narrow layer of myelin and the naked axis cylinders, represent regenerated fibres. The bundle above mentioned can be traced for a distance of about 4 ctm. below the enlargement. They then split up, and the nerve fibres are soon lost in the connective tissue surrounding them. Near the peripheral wound, and in the peripheral part of the resected ulnar, no axis cylinders were seen. In sections through the region of the down-turned flap the collapsed sheaths, containing a small amount of nucleated protoplasm, were found. In these no evidence of regeneration was seen. In this experiment the budding axis cylinders of the central

ulnar segment did not follow the course of the reflected flap, but were lost in the connective tissue surrounding the peripheral end of the central stump.

In the experiments of 147 days' duration (Nos. 47 and 48) the appearances presented on microscopical examination were essentially the same as those described for Exp. 46. The central segment presents a slight enlargement, beyond which extends a bundle of nerve fibres, some of which are medullated, while others are not. At a point about 4 ctm. below the enlargement this bundle breaks up, and the nerve fibres assume a very irregular course, running in all directions, and are finally lost in the connective tissue. In Exp. 47 a few small bundles of nerve fibres were observed in the neighborhood of the peripheral wound, but could not be traced through the wound into the peripheral ulnar segment. In the above experiments sections were made from the different parts of the ulnar below the place of resection, but no regenerated fibres were found. The conclusions reached may be summarized in the following statements:—

I. The nerve flap, whether it be made from the central or peripheral stump of a resected nerve, degenerates throughout its whole extent. The entire peripheral segment likewise degenerates.

II. In that part of the central stump from which the nerve flap was made many degenerated fibres are found, a result of the traumatism incurred while forming the flap.

III. The junction of the flap with the peripheral end of the central stump is not of such a nature as would offer favorable mechanical conditions, such as would best guide the down-growing axis cylinders of the central stump to the peripheral part of the resected nerve. The experiments show that the budding axis cylinders are lost in the connective tissue surrounding the peripheral end of the central segment.

(*e–f*) *Nerve Grafting and Cross-Suturing and Grafting.*

The microscopical examination of the nerves involved in Exps. 49 and 50 corroborate in every way the results obtained at the physiological examination of the nerves involved

in the above experiments, the details of which are given in the second part of this paper. The peripheral median stump, which was stitched (grafted) to the accompanying ulnar (Exp. 49), was found to be completely degenerated, and no new axis cylinders or nerve fibres were to be found in the connective tissue uniting the central end of the peripheral part of the median to the ulnar. The central median stump terminated in a large bulb presenting the microscopical appearance usual for such structures. The ulnar, to which the median was grafted, was in no way altered structurally.

In Exp. 50, in which, it will be remembered, the central part of a resected median was sutured to the peripheral portion of a resected ulnar (the segments exsected having been taken from different levels in the two nerves), the microscopical examination shows the peripheral part of the ulnar to contain newly formed nerve fibres throughout its whole extent. In longitudinal section through the junction of these two nerves, the young nerve fibres found in the peripheral end of the central median stump can be traced through a narrow layer of connective tissue into the peripheral part of the ulnar. We are, I think, justified in seeking the origin of regenerated fibres found in the ulnar in the down-growing axis cylinders of the central stump of the median, which had been sutured to the peripheral part of the accompanying ulnar.

So far this experiment is a confirmation of the results obtained by other observers, and again proves that union with return of functional activity after cross-suturing two spinal nerves is possible. The short segment of the peripheral median, which was grafted to the peripheral ulnar stump, was completely degenerated ; in none of the sections made from it were any new nerve fibres found. The central ulnar segment, stitched to the central median stump, terminated in a large bulb surrounded by connective tissue, by means of which it was united to the median. This tissue was free from naked axis cylinders or new-formed nerve fibres.

Tillmanns' modification of Létiévant's operation of cross-suturing the long ends of two divided nerves having a parallel course, when there is loss of nerve substance to the extent that

an ordinary suture cannot be made, and when the injury occurs at a different level in the two nerves, which modification consists in grafting the short segments of the two divided nerves to the long one, after they have been cross-sutured, would not seem to be of any value, as, I think, this experiment clearly shows.

General Conclusions.

A review of all the experimental work reported by other writers and myself, dealing with the operative treatment of a divided peripheral nerve after loss of substance to the extent that an ordinary nerve suture cannot be made, and of such clinical cases as I have been able to collect, will, I think, warrant the following conclusions :—

I. That it is possible to restore the functional activity to the peripheral part of a divided nerve with loss of nerve substance, if the resected ends are united with a segment taken from some other nerve trunk, with catgut threads, or with a bone-drain or tubular suture.

II. Of all the methods tried (implantation of a nerve segment, tubular suture, suture à distance, nerve flap, grafting the central end of the peripheral stump of a divided nerve to an accompanying uninjured nerve, and cross-suturing long ends and grafting short stumps where two nerve trunks are cut at a different level) the most favorable results are to be obtained after the implantation of a nerve segment, the two ends of which have been sutured with one or several direct catgut sutures to the resected ends of the injured nerve. The favorable results obtained by Assaky in his experiments of suture à distance, and Vanlair after bone-drain suture, are not, I think, exceptions to this statement ; as in their experiments much shorter segments were removed from the nerve operated upon than was the case in my experiments of resecting a nerve and implanting a segment from another nerve trunk, to which fact the somewhat earlier return of function recorded by them can be attributed.

III. The regeneration of the peripheral end (which always degenerates so that only the old sheaths of Schwann, containing

a band of nucleated protoplasm developed from the hypertrophied protoplasm and proliferated internodal nuclei of its fibres, are met with) is the result of an outgrowth of new axis cylinders from the undegenerated axes of the central stump, the budding axis cylinders following the paths of least resistance, through the tissue implanted or supplanting the implanted substance, thus reaching the peripheral part of the resected nerve wherein their growth continues.

IV. Since the fibres in an implanted nerve segment degenerate so that only the collapsed sheaths containing a small amount of nucleated protoplasm remain; and the catgut threads used in a suture à distance, and the bone tube employed in a tubular suture, are almost entirely absorbed before regeneration from the central end has reached any degree of prominence, we must conclude that the implanted substances can serve only as a guide to the down-growing axis cylinders. Any further function cannot be ascribed to them.

V. That the degenerated fibres of an implanted nerve segment offer much more favorable mechanical conditions to the down-growing axis cylinders of the central stump than does the loose connective tissue supplanting the catgut threads or the bone drain in experiments of suture à distance or tubular suture, is shown by the fact that the new nerve fibres have a much straighter course and more regular arrangement in the tissue uniting the resected ends of the injured nerve in such experiments than in the case where the latter procedures are made use of.

VI. The above methods may be used in a primary operation immediately after injury to the nerve, or as a secondary operation in cases when the operation is performed after a complete or partial healing of the wound. The results are quite as favorable in a secondary operation, although the return of function takes place somewhat more slowly.

VII. Making a nerve flap from the central or peripheral stump is, for the following reasons, not to be recommended: (*a*) The flap from the central end degenerates, and its connection with the central end does not prevent this; (*b*) The junction of the flap with the central end is a union not so

favorable as when an implanted nerve segment is carefully sutured to the central stump; in the latter case the fibres of the implanted piece are brought end to end with the fibres of the central end, in the former not; (*c*) The down-growing axis cylinders of the central end bud into the fibrous tissue and not into the reflected flap.

VIII. Regeneration through an engrafted connection between the peripheral part of a divided nerve and an accompanying uninjured nerve trunk is not possible.

IX. In the Létiévant-Tillmanns operation (cross-suturing the long ends of two divided nerves and engrafting the short ends to the accompanying long stump) regeneration of but one of the peripheral segments of the two injured nerves can be hoped for; the other peripheral segment remains degenerated; as there is no reason why a regeneration of both the peripheral stumps may not be attained after the implantation of a nerve segment between the respective ends of the two injured nerves, preference should be given to the latter method.

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EXPLANATION OF PLATE XXXIV.

All diagrams were made with the aid of the camera lucida.

FIG. 1. Shows longitudinal section of the implanted segment two days after operation, fixed in Flemming's solution, and stained in safranin and licht grün. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. The medullary sheath is broken into irregular fragments; *a*, nerve corpuscles; one of which seems to be in the early stages of karyokinetic cell division; *b*, polynuclear white blood cell.

FIG. 2. Cross-section of middle of implanted segment three days after operation. Fixed in Flemming's solution. Stained in safranin and licht grün. Leitz $\frac{1}{12}$, oil immersion, ocular No. 3. The cut ends of the nerve fibres are of irregular shape, varying in size and degree of degeneration. The myelin is stained black, and the protoplasm of the fibres of a reddish color; *a*, a nerve corpuscle; *b*, a nerve corpuscle in karyokinetic division. Large number of polynuclear white blood cells in the spaces between the nerve fibres; *c*, endoneural connective tissue cells; *d*, cell in diaster stage.

FIG. 3. Longitudinal section of implanted segment five days after operation. Hardened in Müller's fluid, and stained in anilin blue. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *a*, fragments of breaking-down axis cylinder stained deeply blue; *b*, nerve corpuscles; *c*, polynuclear white blood cell; *d*, remains of medullary sheath.

FIG. 4. Longitudinal section of implanted segment nine days after implantation. Fixed in Flemming's solution, and stained in safranin and licht grün. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. Sheaths of implanted fibres filled with a faintly granular protoplasm; relatively few nerve corpuscles. The uppermost fibre contains one large and several small fragments of myelin.

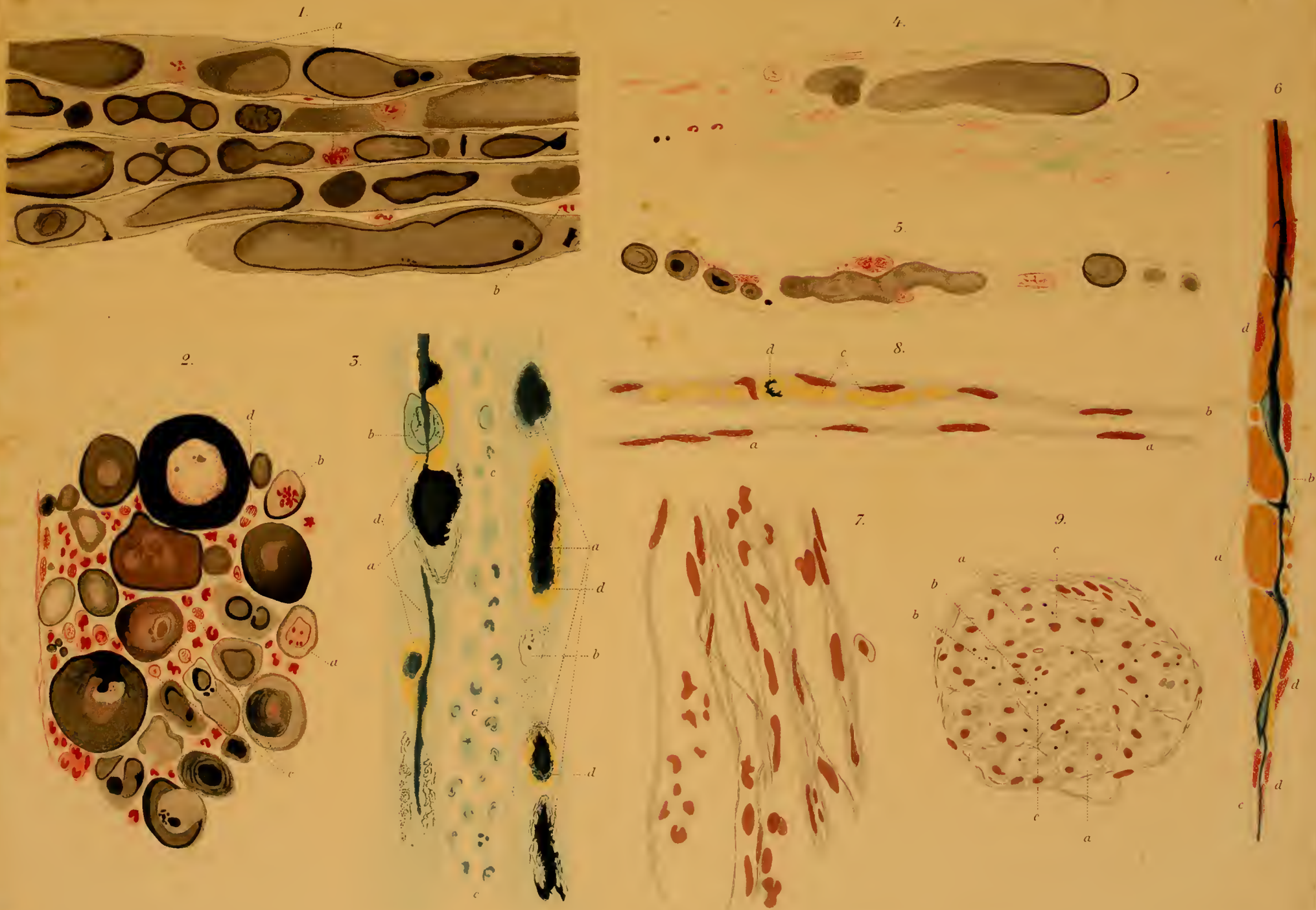
FIG. 5. Degenerating fibre taken from peripheral ulnar one inch below peripheral wound, nine days after cutting the nerve, showing extent of degeneration and proliferation of nerve nuclei. Flemming's hardening. Stained in safranin and licht grün. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3.

FIG. 6. Teased preparation from central wound twenty-one days after implantation. Hardened in Müller's fluid, and stained in anilin blue and safranin. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. The figure shows the distal end of a fibre from central ulnar stump. *a*, enlarged distal end of axis cylinder ending free in embryonic fibre (*c*); *b*, fragments of myelin; *d*, nerve corpuscles.

FIG. 7. Teased preparation from middle of implanted segment twenty-one days after implantation. Hardened in Müller's fluid, and stained in anilin blue and safranin after Stroebe. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. Shows the collapsed sheaths of nerve fibres of implanted segment. Polynuclear white blood cells and connective tissue cells between the fibres.

FIG. 8. Teased preparation from peripheral ulnar twenty-one days after section. Hardened in Müller's fluid, and stained in anilin blue and safranin. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *a*, fibre completely degenerated; *b*, shows remains of medullary sheath (*c*); *d*, ball of myelin containing fragment of old axis cylinder.

FIG. 9. Cross-section of middle of implanted segment thirty-nine days after implantation. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. Shows a large amount of connective tissue between old sheaths and new fibres. *a*, completely degenerated nerve fibres; *b*, developing fibres showing axis cylinder surrounded by delicate sheath; *c*, new fibre in old sheath.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EXPLANATION OF PLATE XXXV.

FIG. 10. Teased preparation from implanted segment thirty-nine days after implantation, taken just below cross-section as shown in Fig. 9. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *a*, old sheaths; *b*, new fibres with delicate axis cylinder stained deeply blue, surrounded by small amount of protoplasm.

FIG. 11. Teased preparation from peripheral ulnar just below peripheral wound thirty-nine days after cutting nerve. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *a*, nerve fibres with axis cylinder; *b*, old sheaths; *c*, old sheath containing a number of balls of myelin.

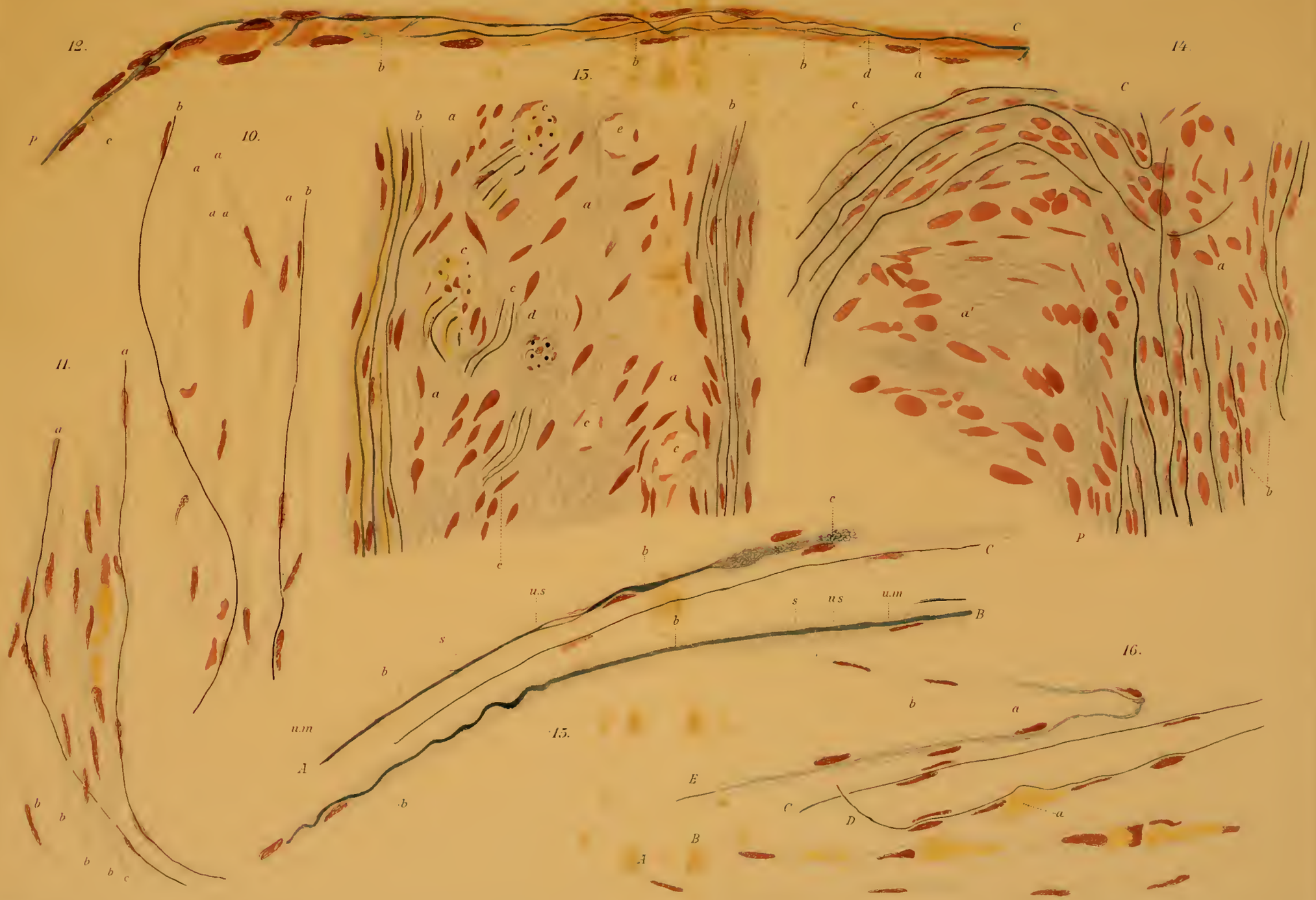
FIG. 12. From teased preparation of peripheral end of central ulnar, one hundred and seventeen days after implantation. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. (*C*) central end; (*P*) peripheral end of fibre as shown in figure. *a*, old axis cylinder from which bud three young axis cylinders; *b*, one of which becomes the axis cylinder of a regenerated fibre at *c*; *d*, node of Ranvier.

FIG. 13. Longitudinal section through central wound (connective tissue between stump of central ulnar and central end of implanted segment) one hundred and seventeen days after implantation. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. Coursing through the interlacing fibrous connective tissue (*a*) are seen a number of small nerve bundles, some of which have quite a regular course (*b*), others a serpentine course, winding their way through the connective tissue (*c*); *d*, a small bundle in cross-section. Some of the nerve fibres are medullated; *e*, small blood vessels.

FIG. 14. Longitudinal section of peripheral wound (connective tissue between stump of ulnar and peripheral end of implanted segment) of dog one hundred and seventeen days after implantation. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *C*, central-ward; *P*, peripheral-ward. *b*, nerve fibres passing quite regularly through connective tissue; *c*, a bundle of nerve fibres evidently deflected in their course by the firmly organized mass of fibrous connective tissue at *a*.

FIG. 15. Three fibres from teased preparation made from small muscular branch passing to flex. carp. ul. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *A*, nerve fibre showing down-growing axis cylinder (*b*) ending in bulbous enlargement (*c*); young fibre contained in old sheath (*s*); *n*, *s*, new sheath; *n*, *m*, newly developed myelin. *B*, showing a similar condition except that peripheral end of down-growing axis cylinder has a wavy course; *b*, axis cylinder; *s*, old sheath; *n*, *m*, newly developed medullary sheath. *C*, new nerve fibre with axis cylinder passing down between the old sheaths.

FIG. 16. Teased preparation from peripheral ulnar at wrist one hundred and seventeen days after cutting the nerve. The figure shows a number of typical fibres in different stages of degeneration and regeneration. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *A*, completely degenerated fibre, no regeneration; *B*, degenerated fibre containing balls of broken-down myelin; *C*, new fibre with axis cylinder and a number of nerve corpuscles; *D*, axis cylinder in old degenerated fibre; *a*, two small fragments of myelin; *E*, fibre showing down-growing axis cylinder ending in slight enlargement at *a*; *b*, continuation of old sheath free from axis cylinders containing a small amount of protoplasm and two nerve corpuscles.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EXPLANATION OF PLATE XXXVI.

FIG. 17. Cross-section of upper end of implanted segment one hundred and fifty-two days after implantation. Müller's fluid hardening. Stroebe's stain. Leitz objective 7, ocular No. 3. Shows cross-section of small nerve bundles varying in size in groups of 3–5, separated by a small amount of fibrous connective tissue.

FIG. 18. Cross-section of lower end of implanted segment one hundred and fifty-two days after implantation. Müller's fluid hardening. Stroebe's stain. Leitz objective 7, ocular No. 3. In this figure the nerve bundles (*a*) are much more scattered than in Fig. 17, separated by a larger amount of fibrous connective tissue.

FIG. 19. Cross-section of peripheral ulnar at wrist one hundred and eighty-two days after implantation. Müller's fluid hardening. Stroebe's stain. Leitz $\frac{1}{12}$ in., oil immersion, ocular No. 3. *a*, completely regenerated fibres with well-developed axis cylinder and medullary sheath; *b*, regenerating fibres, axis cylinder developed but not the medullary sheath; *c*, empty sheaths, probably of old fibres.

FIG. 20. Longitudinal section through tissue supplanting implanted bundle of catgut, one hundred and fifty-two days after implantation. The sketch was made of section taken about 1 ctm. below central wound. Müller's fluid hardening. Stroebe's stain. Bausch and Lomb $\frac{1}{4}$ in. obj., and Leitz ocular No. 3. *C*, central-ward; *P*, peripheral-ward. A number of small nerve bundles are seen passing down between bundles of fibrous tissue. *a*, bundles having a course in line with axis of ulnar nerve in this portion of arm; *b*, a bundle having a wavy course; *c* and *d*, passing in a direction nearly at right angles with the other nerve bundles; *f*, blood vessels. It will be noticed that a relatively large amount of fibrous connective tissue intervenes between the nerve bundles.

FIG. 21. Cross-section through tissue supplanting implanted catgut bundle one hundred and fifty-two days after operation. The section was made about 2 ctm. below central wound. Müller's fluid hardening. Stroebe's stain. Bausch and Lomb $\frac{1}{4}$ in. obj., Leitz ocular No. 3. Many of the connective tissue bundles are seen in cross section (*a*); some in longitudinal or oblique section (*b*); *c*, blood vessels; *d*, small nerve bundles in cross-section; *e*, bundles cut longitudinally in section, must, therefore, have had a course at right angles to longitudinal axis of ulnar nerve in this part of fore-leg.

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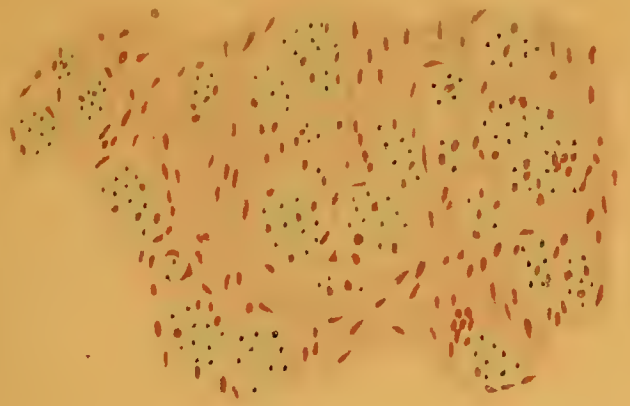

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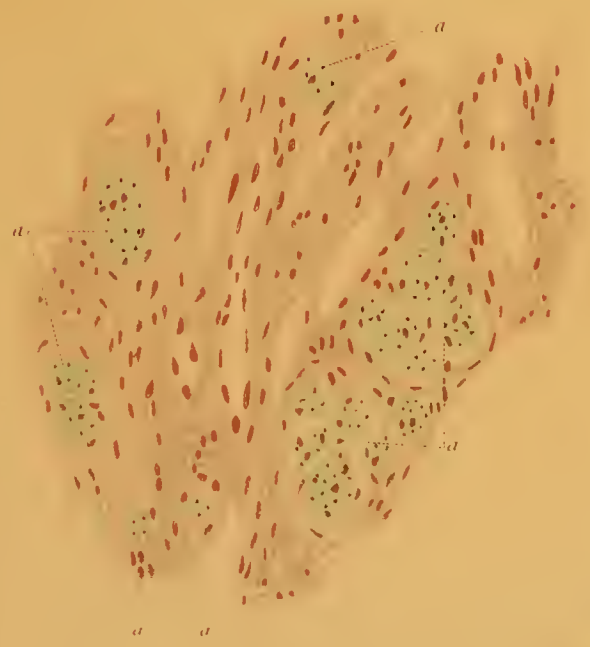
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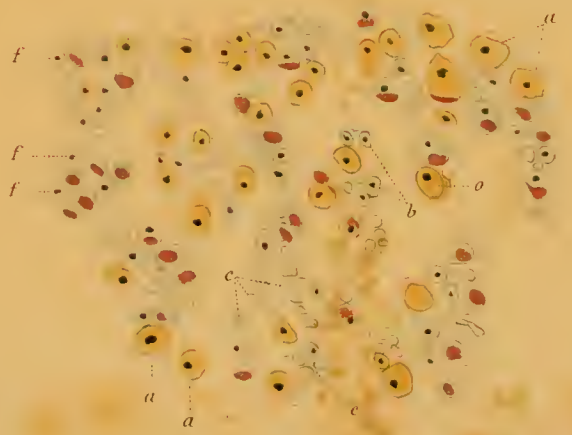
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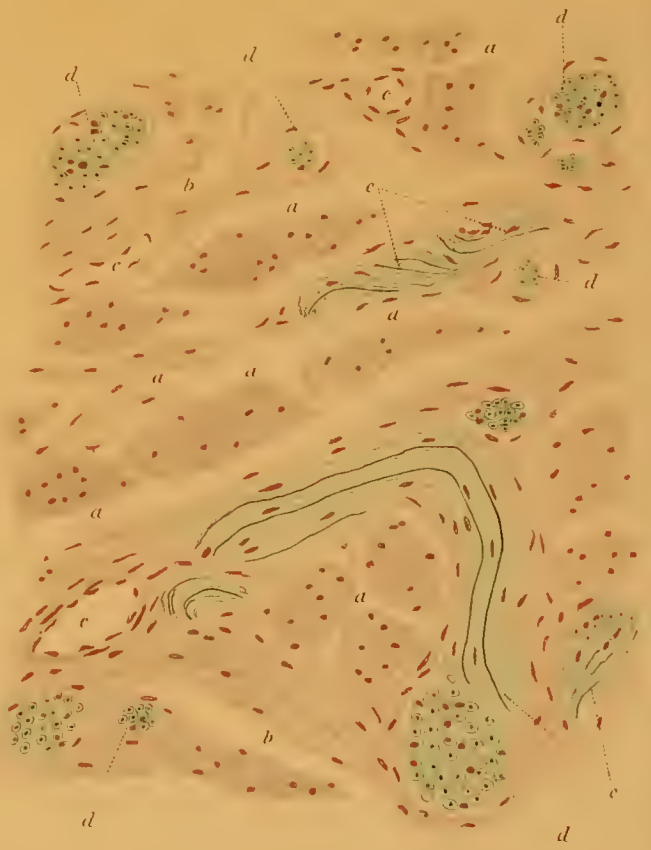
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THE CLEAVAGE OF THE EGG OF VIRBIUS ZOSTERICOLA, SMITH.

A CONTRIBUTION TO CRUSTACEAN CYTOGENY.

FREDERIC P. GORHAM.

THE following investigations were carried on at the Marine Biological Laboratory at Woods Holl, Mass., and at the Biological Laboratory of Brown University, during the years 1893 and 1894.

Unlike the eggs of most Decapods, those of the "green shrimp," *Virbius zostericola*, Smith, are remarkably favorable for the study of early cell-lineage. The thin chorion, the transparency of the yolk substance, the depth of the cleavage-planes and the early invagination, permit the history of the blastomeres to be followed with great accuracy. The dividing nuclei and the direction of the spindles can be seen in the living egg, and if the study of fresh material is supplemented by that of preserved and stained specimens, the result is all that can be desired.

Females bearing eggs in all stages of development (those of a single individual being of the same stage) can be obtained throughout the summer months and eggs removed from the pleopoda of the female will continue their development in watch-glasses, under the microscope, if the water is occasionally renewed. The segmentation and invagination can be watched from beginning to end in a single egg, about eight hours being required for the stages which intervene between the appearance of the first cleavage-plane and the completion of the process of invagination ; nuclear division takes place about once an hour.

No trace of polar bodies has been seen. The early segmentation of the egg is total and regular, and at first it is impossible to distinguish between the animal and vegetal poles. The division of the nuclei precedes the division of the yolk and successive spindles are always arranged at right angles to each other.

The Unsegmented Egg.

The eggs are ellipsoidal, their major axis measuring about 0.36 mm. The nucleus is central, surrounded by a mass of radiating protoplasm which is in turn surrounded by coarsely granular yolk, into which the protoplasmic fibres radiate. The chorion is thin and transparent, and, unlike that of many crustacea, is easily penetrated by killing fluids and stains.

First Cleavage.

In the great majority of cases the first cleavage-plane cuts the egg at its equator, the spindle of the dividing nucleus lying in the major axis (Pl. XXXVII, Fig. 1). Cases are more or less frequent, however, in which the direction of the spindle is considerably inclined to the major axis (Pl. XXXVII, Fig. 2), and in a few cases the direction of the spindle almost coincides with the minor axis of the egg, and the cleavage-plane passes through a meridian (Pl. XXXVII, Fig. 3). Several eggs have been observed in which cleavage-planes did not appear until four nuclei were present ; the first and second planes in such cases appeared simultaneously (Pl. XXXVII, Fig. 4).

Second Cleavage.

Soon after the first cleavage-plane is completely formed the two nuclei again divide. This division takes place in one of two ways, (1) the spindles lying parallel to each other (Pl. XXXVII, Fig. 5), or (2) perpendicular to each other (Pl. XXXVII, Fig. 6). In either case their position is at right angles to that of the previous spindle and, though intermediate positions may occur, the great majority take one or the other of these two directions. In about seventy-five per cent of the eggs the spindles are parallel, while in the remainder they are perpendicular, thus causing two radically different methods of arrangement of the later blastomeres. We shall follow the course of segmentation in each of these cases separately.

Type I. — *The spindles of the nuclei in the 2–4-cell stage are parallel to each other and the "cross-furrows" are perpendicular to each other* (*Pl. XXXVII, Fig. 7*).

In this type of segmentation, after the nuclei have divided, the first cleavage-furrow becomes bent at the points where the second furrow is about to appear and forms two "cross-furrows" which are perpendicular to each other (Pl. XXXVII, Fig. 7). The second plane then appears, not as one continuous meridianal plane dividing the two blastomeres, but the portion dividing one half of the egg lies somewhat inclined to that dividing the other.

While the second cleavage-plane is forming there is a change in the position of the major axis of the egg in relation to the first cleavage-plane. Just before the second plane is formed the major axis is, in most cases, perpendicular to the first plane (Pl. XXXVII, Fig. 7), but as the second plane appears the shape of the egg changes, and the major axis is no longer perpendicular to the first plane, but takes the direction shown in Pl. XXXVII, Fig. 9.

Type II. — *The spindles of the nuclei in the 2–4-cell stage are parallel to each other and the "cross-furrows" lie parallel to each other in the major axis of the egg* (*Pl. XXXVII, Fig. 8*).

The only difference between eggs which develop according to this type of segmentation and those which develop according to Type I is in the position of the "cross-furrows." In this type the "cross-furrows" are parallel to each other and in the major axis of the egg (Pl. XXXVII, Fig. 8), so that the lateral blastomeres are in contact on both sides of the egg (Pl. XXXVI, Fig. 10), instead of the alternate arrangement of Type I (Pl. XXXVII, Fig. 9).

Type III. — *The spindles of the nuclei in the 2–4-cell stage are perpendicular to each other* (*Pl. XXXVII, Fig. 6*).

In this type the planes dividing the two blastomeres are meridianal, but lie at right angles to each other (Pl. XXXVII, Fig. 11). There is no change in the first furrow, or in the direction of the major axis of the egg.

Owing to these differences in the method of segmentation we have three distinct types of arrangement of the blastomeres in the four-cell stage (Pl. XXXVII, Figs. 9, 10, and 11). This variation in the method of cleavage is not due to artificial causes, but takes place under natural conditions in the sea. The various types of cleavage are found in the eggs of a single female, and all finally produce normal embryos. About fifty per cent of the eggs develop according to Type I (Pl. XXXVII, Fig. 9), about twenty-five per cent according to Type II (Pl. XXXVII, Fig. 10), and the remaining twenty-five per cent according to Type III (Pl. XXXVII, Fig. 11).

One is not justified in claiming that certain blastomeres of eggs of different types of segmentation are equivalent, for there are no distinguishing marks whereby the blastomeres can be identified. The eggs are all regular in outline, and the several blastomeres do not vary in size or color, and in Types I and II it is impossible to distinguish one apical or one lateral cell from the other, and in Type III all four blastomeres are *exactly* alike.

THIRD CLEAVAGE.

Type I. — After the egg is completely divided into four blastomeres the nuclei again divide in such a manner that their spindles are all parallel to each other, and perpendicular to the major axis of the egg (Pl. XXXVII, Fig. 12). The third cleavage-plane is meridianal, and cuts the four blastomeres at right angles to the first and second planes (Pl. XXXVII, Fig. 12).

Type II. — The third cleavage in this type is essentially the same as in the previous type, but the cells derived from the lateral blastomeres of the four-cell stage are in contact on both sides of the egg (Pl. XXXVII, Fig. 13).

Type III. — In this type the third process of cleavage results in the formation of four spindles, of which the two in each half of the egg are parallel to each other, but perpendicular to the two in the other half of the egg (Pl. XXXVII, Fig. 14). The four blastomeres are then divided meridianally, the new planes appearing as direct continuations of the planes of the second cleavage (Pl. XXXVII, Fig. 14). The eight blastomeres

into which the egg is divided, it will be seen, are arranged quite differently from those of Types I and II.

Fourth and Fifth Cleavage.

In all three types of segmentation the blastomeres continue to divide, always in a direction at right angles to their former division, until there are thirty-two blastomeres. The nuclei now occupy positions at the surface of the egg, all of the spindles in the 16–32-cell stage taking a direction tangential to the surface.

Sixth Cleavage.

In passing from thirty-two to sixty-four blastomeres there is the first indication of invagination. The thirty-two spindles form in the normal way, and twenty-eight of the blastomeres are divided by cleavage-planes. In the four remaining blastomeres no cleavage-planes appear, and these four cells soon begin to invaginate (Pl. XXXVII, Figs. 18, 19, and 20). In the thirty-two and sixty-four-cell stages the cleavage-planes do not extend to the center of the yolk, and the four nuclei which sink below the surface are no longer separated by distinct cell-walls.

When we trace back the history of the invaginating cells of the three types of segmentation we find them arising from different parts of the egg, and by entirely different methods of segmentation. If we regard these four cells, then, as equivalent cells in each of the three types, we must conclude that in the egg of Virbius the prospective value of the cells is not at all a "function of their position," but that the value of each cell is determined, as early as the two-cell stage, by some process of qualitative division of the nuclei.

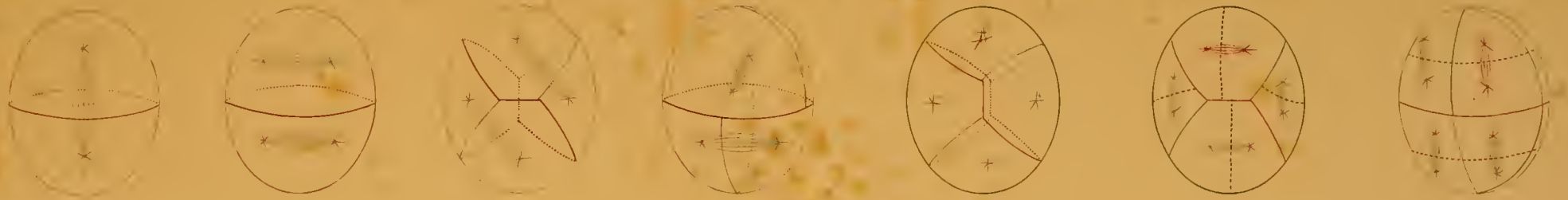
Brown University, March 1, 1895.

EXPLANATION OF PLATE XXXVII.

In all the figures the *red* line represents the first cleavage-furrow, the *blue* line the second, the *green* line the third, the *broken black* line the fourth, the *continuous black* line the fifth, and the *dotted black* line the sixth. The *red* nuclei ultimately invaginate.

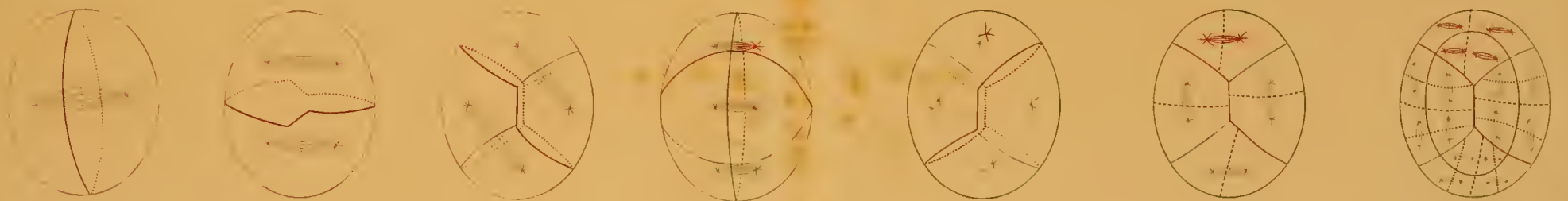
FIG. 1. 1–2-cell egg, spindle in major axis.
FIG. 2. 1–2-cell egg, spindle inclined to major axis.
FIG. 3. 1–2-cell egg, spindle nearly in minor axis.
FIG. 4. 2–4-cell egg, first and second cleavage-planes appearing simultaneously.
FIG. 5. 2–4-cell egg, spindles parallel.
FIG. 6. 2–4-cell egg, spindles perpendicular. Type III.
FIG. 7. 2–4-cell egg, spindles parallel and "cross-furrows" perpendicular. Type I.
FIG. 8. 2–4-cell egg, spindles parallel and "cross-furrows" parallel. Type II.
FIG. 9*a*. 2–4-cell egg. Type I.
FIG. 9*b*. Opposite side of same egg.
FIG. 10*a*. 2–4-cell egg. Type II.
FIG. 10*b*. Opposite side of same egg.
FIG. 11. 2–4-cell egg. Type III.
FIG. 12*a*. 4–8-cell egg. Type I.
FIG. 12*b*. Side view of same egg.
FIG. 12*c*. Opposite side of same egg.
FIG. 13*a*. 4–8-cell egg. Type II.
FIG. 13*b*. Side view of same egg.
FIG. 13*c*. Opposite side of same egg.
FIG. 14. 4–8-cell egg. Type III.
FIG. 15*a*. 8–16-cell egg. Type I.
FIG. 15*b*. Opposite side of same egg.
FIG. 16*a*. 8–16-cell egg. Type II.
FIG. 16*b*. Opposite side of same egg.
FIG. 17. 8–16-cell egg. Type III.
FIG. 18. 32–64-cell egg, Type I, showing four cells which invaginate.
FIG. 19. 32–64-cell egg, Type II, showing four cells which invaginate.
FIG. 20. 32–64-cell egg, Type III, showing four cells which invaginate.

I. V. IXa XI. XIIIa. XVa. XVII.



II VI. IXb. XIIa. XIIIb XVb. XVIII.

III VII. Xa. XIIb. XIIIc. XVIa. XIX.



IV. VIII Xb XIIc. XIV. XVIb XX