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STUDIES IN THE DEVELOPMENT OF THE OPOSSUM DIDELPHYS VIRGIANA L.

I. HISTORY OF THE EARLY CLEAVAGE

II. FORMATION OF THE BLASTOCYST

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Contribution from the Zoological Laboratory of the University of Texas, No. 126

SIX TEXT FIGURES AND ELEVEN PLATES

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INTRODUCTION

a. *Statement of the problem*

The desirability of increasing our knowledge of early marsupial development has frequently been emphasized by students of mammalian embryology. The almost yolk-free condition of the Eutherian egg, with its consequent holoblastic type of cleavage, is believed to have developed secondarily from a telolecithal and distinctly meroblastic ovum. This latter condition obtains in the monotreme egg, which may be considered transitional between the Sauropsida and the two higher classes of Mammalia. The large yolk-mass and the shell of the marsupial egg as well as other considerations make it highly probable that the Marsupials are intermediate between the Monotremes and the true Placentalia. It is reasonable, therefore, to expect the study of the marsupials to contribute to the solution of many problems in mammalian embryology which have arisen from the already very extensive study of Eutherian development.

Observations on the early development of marsupials have been very few. Up to 1910, when Hill published his excellent

account of the cleavage of the egg of Dasyurus, our knowledge of the early stages of marsupials rested largely on Selenka's ('87) work on the opossum. Because of the prominence of this author's famous 'Studien, in which the account was published, Caldwell's ('87) interpretation of the outer envelope of the marsupial egg as the shell membrane was completely overlooked until recalled by Hill. Inasmuch as his illuminating results cast doubt upon the validity of Selenka's interpretations, as well as the accuracy of his descriptions, which seemed to be based upon insufficient material, it seemed desirable to study the American marsupial in this locality where sufficient fresh and normal material could be secured. The problem was suggested to the writer by Dr. J. T. Patterson and the work was done under his direction. I am pleased gratefully to acknowledge his helpful suggestions throughout the progress of the work.

b. *Review of the literature on the early history of the marsupial egg*

1) *The work of Caldwell and Selenka.* In 1883–4 Caldwell collected a considerable amount of early marsupial and monotreme material in Australia. In 1884 he published several preliminary communications in which he recorded the discovery of the yolk mass of the monotreme egg. This discovery greatly increased Selenka's interest in marsupial embryology, in the study of which he had been for sometime engaged when Caldwell's paper appeared. Caldwell must also be credited with the discovery of the 'shell' of the marsupial egg; for he correctly homologized the "thin transparent membrane, 0.0015 mm. thick," of the marsupial, Phascolarctus, with the shell membrane of the monotreme egg. This interpretation Selenka completely ignored, for he mistook the shell membrane of the opossum for the corona radiata.

After several years of fruitless experiments in attempting to rear Australian and Brazilian species, Selenka imported a hundred or more individuals of the 'hardy' North American species, Didelphys virginiana, two females of which furnished the 16 eggs that formed the basis of his account (Selenka, '87) of cleavage and blastocyst formation.

He found that cleavage begins almost exactly 5 days after copulation (compare Hill and O'Donoghue's 'post-oestrus' period[1]) and that birth takes place 7 days and 20 hours later. The author states that all of the 7 to 27 eggs or embryos found in any one animal are practically in the same state of development. No external indication of pregnancy, no changes even in the mammary glands, were to be noted until near the time of parturition.

According to Selenka, fertilization takes place in the upper part of the oviduct and cleavage begins in the uterus, the eggs scattering and remaining for three days floating in the uterine fluid. Maturation was not observed, although sections of ovaries were made and nine eggs were taken from the Fallopian tube.

Except for a few unessential details, Selenka correctly described the unsegmented egg of the opossum. His specimens were for the most part shrunken, as indicated by the shape of the ova and the 'perivitelline space' between the zona pellucida and the albumen layer, a condition I have not found in well-fixed normal eggs.

The first of Selenka's two specimens yielded one 2-celled and one 20-celled uterine egg and nine unfertilized eggs from the oviduct, all badly shrunken and of little value for study. It is interesting to note, however, that the 2-celled egg showed some elimination of yolk and that the 20-celled stage was practically a young blastocyst and contained an included ('entoderm') cell.

The second specimen furnished eggs in the following stages: one each in the 4-, 8,- 42- and 68-celled stages respectively; 2 young blastocysts containing some entoderm; and 8 advanced blastocysts ranging from 1.05 to 1.43 mm., the largest showing a layer of mesoderm in the embryonic area.

The blastomeres of the 4-celled stage were conical in form and of equal size and structure. They are radially arranged about a cleavage cavity. The 8-celled stage is made up of two superimposed rings of four cells each. From these two specimens Selenka concluded that the blastomeres of the 4-celled stage

[1] Hill and O'Donoghue ('13), p. 145.

maintain their radial arrangement, that is, they do not shift as is the case in the Eutherian egg. With these two stages before him the author could not avoid the conclusion that the third cleavage is horizontal, and in view of polar differentiation of his next, or 42-celled stage, he expresses the opinion that the third cleavage is qualitative, dividing the four blastomeres into an upper ring of ectodermal and a lower ring of entodermal cells.

The young 'gastrulae,' as Selenka calls the 42– and 68-cell stages, are unilaminar blastocysts in which a polarity is to be noted, in that the cells at one pole are larger and more yolk-laden than those of the other pole. The larger cells Selenka considered entodermal, i.e., lineal descendants of the lower ring of the 8-celled stage just described. In each case there was found within the blastocyst cavity a large included cell, which the author called the 'Urentodermzelle.' Hence the implication is that the entoderm is formed from the lower half of the blasto-cyst and from cells included within the cavity. Such an inter-pretation will be shown in this paper to be incorrect, as was already suggested by Hill in his study on the egg of Dasyurus.

The next blastocysts described by Selenka evidently suffered greatly in preparation and were drawn rather diagramatically; hence I am unable to make any helpful comparisons between them and my own specimens. No light is thrown upon the manner of entoderm formation by these two specimens.

The other four blastocysts figured by Selenka are older, as indicated by the progress in the absorption of the albumen. The wall of the youngest of these is didermic for two-thirds of its surface area. In the oldest of the four, which is 1.45 mm. in diameter, the mesoderm and the primitive streak are already laid down. The six blastocysts intermediate between these two are fully formed didermic specimens and were faithfully described by Selenka. It is these blastocysts with the entoderm almost or quite completed of which Hill says: "Of Selenka's early mate-rial, I think it is these blastocysts alone which had any chance of giving origin to normal embryos."[2] It is my judgment that

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[2] Hill ('10), p. 11.

the early blastocysts also represent normal stages of the opossum embryos. With so little material before him, some of which was indeed not entirely normal, it is no wonder that Selenka should have wrongly interpreted in every essential detail the cleavage of the opossum egg.

Mention should be made in passing of a short paper by Minot ('11) in which the late bilaminar blastocyst of the opossum is described in some detail. The article, however, adds little, if anything, to the work of Selenka, which it entirely ignores. Indeed, the author fails to recognize the shell-membrane, as did Selenka, and in his interpretation of Hill's ('10) work falls into other minor errors. The article by Dr. Minot will be discussed in Part III of these studies, to be published in a subsequent number of this Journal.

2) *Cleavage and blastocyst formation in Dasyurus as described by Hill '10).* Inasmuch as Professor Hill's thorough study of the Australian 'native cat' furnished a strong impulse for the study of the development of the opossum and since comparisons will be made in this paper between the two forms, it is deemed desirable to review in some detail the developmental processes as described by the British author.

The ovarian ovum of Dasyurus, aside from its much greater size and larger amount of yolk as compared with Eutherian ova, exhibits nothing unusual before the period of maturation. During the period of growth the ovum is more or less homogeneous throughout, or perhaps somewhat centrolecithal. But at the time of formation of the first polar body, which is given off in the ovary as in the Eutheria, the egg takes on a striking polarity through the accumulation of surplus yolk at the animal pole of the egg. The egg maintains this condition during its passage through the oviduct, in which it adds the albumen layer and the shell and where insemination takes place. The pronuclei and the first cleavage spindle lie in the granular formative cytoplasm, which occupies about two-thirds of the egg and which appears in longitudinal section as a broad crescent, the two horns of which partly envelop the yolk mass (text fig. 4A, see page 34).

The first cleavage plane is, of course, meridional and in this first division the cytoplasm of the two blastomeres round up in such a fashion as to cast off bodily the entire polar mass of yolk. The second cleavage plane which is also meridional, passes at right angles to the first, leaving the yolk body partly enclosed by the upper, more pointed apexes of the four blastomeres. These are, therefore, radially arranged and do not shift their position, as do the blastomeres of Eutheria in the corresponding stage. The third cleavages are again meridional, dividing the egg into eight equal blastomeres arranged in an open ring near or a little above the equator of the egg. Each cell again exhibits a well-marked polarity, both in greater deutoplasmic content of the lower pole as compared with the upper, and in the presence of the nucleus somewhat toward the upper pole.

In view of the subsequent history of the egg the author is fully justified in calling the upper the formative and the lower the non-formative pole of the egg; for the fourth cleavage is horizontal and divides the eight blastomeres quantitatively and qualitatively into an upper ring of eight, smaller, lighter-staining cells and a lower ring of larger, more darkly staining cells. All of these facts are fully evidenced by a set of superb photomicrographs. According to the author's interpretation, the upper ring of cells is destined to form the embryonic portion of the blastocyst; the lower, the non-formative portion.

For a time the rapidly multiplying cells of each ring occupy a more or less clearly horizontal position somewhat above the equator of the egg. They soon reach the periphery, however, that is, they become applied to the inner surface of the shell, and the formative cells proliferate toward one pole, the non-formative cells toward the opposite pole of the egg. The cells finally meet at the poles, and thus complete the unilaminar blastocyst. By this time the entire albuminous layer has disappeared, but considerable yolk still remains in the cavity. During cleavage the egg grows from about 0.35 mm. to about 0.45 mm. in diameter and the shell becomes somewhat thickened (text fig. 4 G).

Following this stage there is a period of growth during which the blastocyst maintains the unilaminar condition. The polar differentiation previously noted now disappears, for the cells of the entire blastocyst are found to have the same structural appearance both in surface view and in section (text fig. 4 *I*).

It is not until the blastocysts have attained a diameter of 3.5 mm. that the polarity again becomes evident structurally. At this time the cells of the formative ectoderm become differentiated, and a little later the entoderm formation begins. The upper formative and lower non-formative areas, of which the latter is usually the larger, are soon separated by a 'sutural line,' The formative cells on one side of the line are usually larger and more lightly staining; those below the line are smaller, more uniformly distributed and possess dark perinuclear zones.

The next stage is that of entoderm formation, which occurs in blastocysts a little more than 3.5 mm. in diameter. The entoderm arises by a proliferation of modified cells of the formative entoderm, the 'entodermal mother-cells.' These appear at various points in the formative region and seem both to migrate out of their position in the epithelial layer of the entoderm and then multiply, and to maintain their position and give off entodermal cells by proliferation. These cells as well as the daughter cells differ markedly in their darker staining qualities from the surrounding ectodermal cells. The young entodermal cells send out pseudopodia-like prolongations, which unite and form a fenestrated entodermal layer. This later closes up to form the primitive entoderm, which spreads until it lines the entire blastocyst cavity.

It should also be noted that the author homologizes the non-formative portion of the marsupial blastocyst with the trophoblast of the Eutheria, the embryo of the former, therefore, being without trophoblastic covering.

The work by Professor Hill here reviewed is the first connected account, supported by a sufficient number of stages, of the early development of a marsupial. It renders Selenka's views untenable and makes questionable some of the stages found by him.

Briefly stated, the present paper, together with one soon to follow, will show that the development of Didelphys differ from that of Dasyurus, first in its indeterminate type of cleavage, and second in the delay of structural evidences of polarity (aside from the clue offered by the position of the polar bodies) up to the time of entoderm formation. Agreement is found in the presence of large quantities of yolk in the egg and in the manner of entoderm formation. Other points of minor importance will be discussed in the body of the paper.

c. *Material and technique*

1) *Material.* The specimens of opossum eggs and blastocysts on which the present study (as well as two other articles shortly to appear) is based, represent collections made during three seasons, but especially during the winters of 1913–1914 and 1914–1915. During the first season, 1912–1913, the collections made resulted chiefly in establishing the breeding season of Didelphys for this locality. About 140 females were used, some of which were killed from time to time during the first season. The large number of specimens required to yield the desired stages is due, in part, to certain difficulties which were at first experienced in breeding the animals in captivity. It is my intention to say more concerning this matter in a subsequent publication on the physiology of reproduction of the opossum.

The present account deals with the unsegmented eggs, cleavage stages and blastocysts taken from twenty different animals. This number includes Female No. 112 which furnished only unfertilized and degenerating eggs; but the specimen is here added for a special purpose, as will appear below. 415 eggs were removed from the uterus or the oviduct of these animals, making an average of 22 eggs from each animal. The extremes in number were 1 and 45. No. 76 yielded 10 eggs from the oviduct on one side only and No. 144 yielded 11 from a single uterus; No. 117 discharged 43 eggs from the right ovary exactly one month after the left ovary had been removed.

Of these 415 eggs 230 were cut into serial sections five or six
micra thick, mounted and stained. A few were mounted in
balsam; some were lost in the handling and a few have been
retained in 80 per cent alcohol.   Perhaps one-sixth or one-seventh
of the eggs taken from inseminated animals were clearly unfer-
tilized and showed signs of degeneration.

Besides these, eggs were removed from 37 other females, so
that about one thousand ova were handled in the progress of
the work.   In all of these last mentioned cases the eggs were
unfortunately degenerate, not being fertilized, and were therefore
usually not counted but discarded as worthless.   Foetuses or
pouch young were secured in several dozen cases.

Old degenerating eggs can easily be recognized.   They are
large and the shell is opaque and covered with a white incrusta-
tion.   Later the shell collapses and the egg becomes a shapeless
mass which sooner or later leaves the uterus.   Young degenerate
eggs in which little change can be noted in the shell or in the albu-
men may usually be recognized by the flattening of the ovum in
the center.   In side view the protoplasm (in which degeneration
first becomes apparent) assumes the shape of a crescent, to be
seen especially well in alcoholic specimens fixed in osmic acid
mixtures.

A detailed account of each animal furnishing the eggs de-
scribed in this study follows.   Unless the time of capture is indi-
cated it is understood that the animals were bred in captivity.
The captives were kept in cages from a few weeks to two months
or more.   Nos. 40 to 58 were secured in 1914; Nos. 76 to 144 in
1915.

2) *History of animals.*   No. 21.   Copulation January 3; killed Janu-
ary 6; ovarian eggs only.
No 40.   Placed with male January 31; killed February 6; 11 eggs;
unilaminar blastocysts with formation of entoderm; three eggs unfer-
tilized.
No. 43.   Placed with male January 28.   Male removed February 2;
killed February 7; 29 eggs: several late unilaminar blastocysts, mostly
later bilaminar blastocysts, 0.8 mm. to 1.0 mm. in diameter; 6 large
unfertilized eggs.
No. 46.   Killed February 8; ovulation recent, as indicated by promi-
nent and blood-shot stigmata on young corpora lutea; 21 small eggs;

3 in the 2-celled stage, 2 in the 4-celled stage, 2 in the 5-celled stage, 7 unfertilized, the remainder unaccounted for.

No. 50. Placed with male February 1; removed male February 2; replaced male February 5; killed February 10. 24 eggs: two 2-celled, two 4-celled, 10 blastocysts of about 50 cells, 2 unfertilized, 8 unaccounted for.

No. 52. With male February 4 to 6; killed February 10. 25 young (very small) eggs: one 4-celled stage; 3 in first cleavage; 14 unsegmented, 6 of which show pronuclei; the remainder unaccounted for.

No. 54. Killed February 13. About 25 small eggs: one 2-celled stage; one 4-celled stage; 2 in first cleavage; 11 undivided, of which 5 show pronuclei; 1 abnormal; 1 egg contained only shell and albumen, the place of ovum being taken by a mass of epithelial cells. Other eggs from this batch showed a number of epithelial cells from the oviduct. The remainder lost and unaccounted for.

No. 55. With male February 6; copulation probably February 7; killed February 13. 22 eggs: all advanced, bilaminar blastocysts 0.8–0.9 mm. in diameter, except 6 unfertilized eggs.

No. 56. Killed February 14. About 20 eggs removed from the Fallopian tubes, all devoid of albumen layer and shell; that is, just as passed from the ovaries.

No. 58. With male February 4; isolated February 8; killed February 15. Very young fresh corpora on ovary. Small, thin-shelled eggs, all unsegmented and unfertilized, but normal and just passed into the uterus.

No. 76. Caught between January 13 and 15; killed January 16. Both ovaries had discharged their follicles, but eggs were found in only one oviduct. These eggs, 10 in number, were almost exactly like those from No. 56.

No. 81. Caught January 18 and killed next day. About 20 eggs: all 4-celled with the exception of 3 or 4 unfertilized eggs.

No. 82. Caught January 18 and killed next day. About 25 eggs: almost fully formed bilaminar blastocysts with the exception of 6 unfertilized eggs.

No. 83. Caught January 18 and killed next day. About 9 eggs: 1 unsegmented; four 4-celled stages exactly like those of No. 81; 3 blastocysts of about 50 cells, like those of Nos. 50 and 88.

No. 85. Caught January 18 and killed the next day. About 25 eggs: one each in the following stages: 6, 7, 9, 10, 12, 14, 15, 17, and 18 cells; three of 8 cells; five of 16 cells and one abnormal egg.

No. 88. Caught about January 18 and killed January 20. Number of eggs not recorded, but 27 were sectioned and mounted, all young blastocysts of 30–55 cells, except 6 abnormal eggs.

No. 94. Killed January 21, several days after capture. A single advanced bilaminar blastocyst, like those of Nos. 43, 55, and 82 was taken.

No. 112. Secured in December. February 11, successful laparotomy performed, corroborating previous diagnosis of pseudopreg-

nancy; recovery perfect.   March 2, all external indications of pseudo-
pregnancy disappeared.   March 14, approaching ovulation indicated.
March 19, 2 p.m., left uterus removed and 20 eggs found.   Animal was
killed at 8 p.m. and 17 eggs recovered from the right uterus.   Eggs
varied in size and were in different stages of disintegration; but the
case is here cited because one egg showed a '4-celled' stage, abnormal
as I am convinced, but comparable in a way with the 4-celled stage of
the opossum as figured by Selenka and with the normal 4-celled egg
of Dasyurus.

No. 117.   February 13, the female having shown external signs of
ovulation, the left ovary and uterus were removed.   20 degenerate
eggs were found.   By March 2 all external signs of pseudopregnancy
had disappeared.   March 17, the sexual period being again indicated,
animal was killed and 43 eggs, small in size, were removed from remain-
ing uterus.   The eggs included the following stages: twelve unsegmented
(several of these showed signs of beginning disintegration); two 2-celled;
two 4-celled (2 of these were apparently abnormal); one 5-celled; two
6-celled; two 8-celled (one of these had seven cells in mitosis); two
13-celled; one 14-celled; one 15-celled; three 16-celled.

No. 144.   February 20, signs of pseudopregnancy.   March 16, signs
of second (or third?) sexual period approaching.   March 20 killed.
11 eggs removed from one uterus only and over-fixed in Carnoy's
fluid: one 12-celled stage, other eggs are young blastocysts like those
of Nos. 50, 82, and 88, or somewhat more advanced.

3) *Securing the eggs.*   On opening the body cavity of the female
the ovary was first examined, from the condition of which the
time that has elapsed since the discharge of the eggs may be
roughly estimated.   Prominent blood-shot stigmata indicate re-
cent ovulation; a smooth surface with deep-seated dingy-yellow
corpora shows that ovulation was not recent.   If in the latter
condition the uterus lacks fullness and turgidity, old degenerate
eggs are likely to be found.

To secure the eggs the uterus is placed in a shallow vessel
containing Ringer's solution at 40° C. and the muscular wall is
slit longitudinally.   The intrauterine pressure at once forces out
the enormously swollen mucosa, which is richly supplied with
blood vessels and convoluted uterine glands.   The uterine mucosa
is next gently pulled apart with two pairs of forceps, exposing
the lumen of the organ, and in the folds of the mucosa the eggs
may easily be seen with the aid of the binocular microscope,
and picked out with a pipette.   Old, encrusted eggs can easily
be seen with the naked eye.   Blastocysts of about 0.8 mm.

diameter are harder to see than the much smaller unsegmented eggs, for the former are perfectly hyaline and the latter possess a white core, the egg proper, surrounded by the transparent shell and albumen layer. After picking out all of the eggs which can be found by a search among the folds of the mucosa, the uterus is turned inside out and shaken in the dish of Ringer's solution. In this way usually several eggs are recovered which would otherwise be overlooked. A few cubic centimeters of fixing solution, such as Bouin's, added to the solution will facilitate the finding of eggs in the bottom of the dish. They can be located by means of the peculiar way in which they roll when agitated by a gentle stream from a pipette, for the eggs differ in this respect from tissue fragments which collect at the bottom of the dish.

Eggs were forced out of the Fallopian tubes by means of a stream of Ringer's solution sent through the tube with a fine-pointed glass pipette.

Aseptic operation was resorted to in about twenty cases in the hopes of securing two stages from the same animal. As opossums have a uterus duplex, they are well adapted for such experiments, for the removal of the uterus and the ovary of one side is a comparatively simple matter. The plan has the obvious advantage of enabling one to note the time of development with accuracy. If unfertilized eggs are found, moreover, the animal may be allowed to come into heat a second time, which happens about one month after the first. Recovery is prompt, and the wound usually heals perfectly.

Unfortunately, in these experiments unfertilized eggs were found in most cases at the time of the first operation. Thus from female No. 109, the left uterus was removed 6 days and 4 hours after copulation and 23 eggs recovered. Six hours later the other uterus was removed and 22 eggs recovered. Both lots, however, proved to be unfertilized and the eggs had begun to break up. Greater fortune, however, attended the study of female No. 114. This animal was operated on 5 days and 4 hours after copulation. The eggs had just been shed, as indicated by fresh stigmata on the ovary, but the eggs were not found, prob-

ably having been lost in the body cavity. Ninety-two hours later the animal was killed and embryos in the eleven somite stage were found in the remaining uterus. Female No. 117 was operated February 13, and 20 degenerate eggs were taken from the left uterus. On March 17, the remaining uterus furnished 3 eggs. The ovary was much enlarged and densely studded with large corpora lutea. The eggs were mostly fertilized. In the remaining cases, even where copulation was not observed, eggs were nearly always found, both after the first and the second oestrus periods, but were unfertilized and hence worthless.

Nos. 81 to 88 were killed in the field, where aseptic operation was impossible.

4) *Fixing and staining.* Four fixing fluids were most often used: Carnoy's, Bouin's, Fleming's and Hill's. Carnoy's fluid gave only fairly good results. Eggs fixed in Bouin's solution always proved usable: shrinkage does not occur, but the cell-elements are not so well fixed as with Hill's mixture. Fleming's solutions, both weak and strong, fix beautifully but cause shrinkage of the egg. The larger blastocysts invariably collapse in this fluid. I have found Hill's picro-nitric-aceto-osmic mixture to be a perfect fixing fluid for the opossum egg; it penetrates the tough shell of the egg rapidly and fixes the cell structures without causing shrinkage. The osmic acid stains the fat granules black in this as efficiently as does Fleming's solution. Hill's solution is made as follows: Mayer's picro-nitric acid, 96 cc., 1 per cent osmic acid 2 cc.; glacial acetic acid, 2 cc. Some specimens of every batch of eggs, except those of No. 144, were fixed in this last mentioned mixture.

It is important that the eggs be carried up into the higher percentages of alcohol by degrees to prevent collapse of the egg envelopes. Since the shell prevents penetration, this envelope may advantageously be removed with fine pointed needles. In the case of older blastocysts a hole may be punched in one side.

Toto preparations were stained in Delafield's haematoxylin and sections in iron-alum haemotoxylin; the latter were counterstained with orange G or eosin. A few sections of blastocysts were stained in safranin.

In order to determine with accuracy the arrangement of the blastomeres in 4- to 16- celled stages, wax models were made after the method of Born.

MATURATION AND FERTILIZATION

a. *The mature ovarian ovum*

A detailed account of the ovarian egg is not now attempted. It is deemed desirable, however, to determine two points: first, the time at which the first polar body is given off, and second, the distribution of yolk in the ovarian egg.

In the case of two females studied, ovulation had not yet taken place three days after copulation. The Graafian follicles were tensely filled with liquor folliculi and bulged out like great glassy beads from the surface of the ovary. The sectioned ovaries were found to contain ripe eggs (fig. 2), as was to be expected; but younger and much smaller follicles taken from other females also contained eggs which had given off the first polar body. Hence, the first maturation takes place some days before ovulation. More exact data cannot be given at this time.

The ripe ovarian egg is broadly elliptical in form and measures in section on the average 0.165 by 0.135 mm. It is surrounded by a well-defined zona pellucida, within which the flattened polar body is found. The polar body is given off at one pole of the elongated egg or at the equator, or it may indeed be seen at any other point on the margin of a section passing through the long axis of the egg. The germinal vesicle of the egg has not yet re-formed preparatory to the second maturation, for the chromosomes are very short rods arranged in the form of an open ring. They lie in the cytoplasm just beneath the surface of the egg and usually to one side of the polar body (fig. 2).

Aside from the position of the polar body there is no other structural evidence of polarity in the ripe ovarian egg of the opossum. Of the many eggs studied in sections none showed any massing of yolk toward one pole such as is strikingly the case in the egg of Dasyurus. This lack of polar concentration of yolk in the mature egg of the opossum is the first important

difference to be noted between the Australian and the American species. Further details of the ripe ovum will be given in the next section which deals with the freshly discharged eggs.

b. *The tubal ovum*

The eggs of the two lots (Nos. 56 and 76) removed from the oviduct agree in all essential respects. They had apparently just entered the oviduct, for only a mere trace of albumen had been laid down on the surface of the eggs. The eggs are normally shed simultaneously from a given ovary and both ovaries discharge their eggs almost if not quite at the same time. In the case of No. 76 the eggs from one ovary were a trifle late and were lost in the body cavity, for, though discharged, they were not to be found in the oviduct.

The eggs discharged in the second or third oestrus period of any given animal, however, show greater variation in size and age. In one case an egg was found in the oviduct, while its fellows had already been for some time in the uterus. The ovaries of animals in the second oestrus usually contain some large unruptured follicles along with fresh corporea lutea. Hence, it is apparent that the discharge of eggs in the second oestrus is not as regular as in the first. The opossum has only one sexual season a year and normally (i.e., in the wild state) only a single oestrus period.

The tubal eggs are usually elliptical, rarely oval or spherical in shape (figs. 1 and 3), as seen both in the fresh state and in most sections. They measure on the slide about 0.137 by 0.115 mm., but range from 0.135 by 0.12 to 0.098 by 0.086 mm. The eggs of batch No. 76 are a little the larger, measuring on the average 0.146 by 0.123 mm. against 0.127 by 0.106 mm. for those of batch No. 56.

The egg is surrounded by a 'rather well-defined homogeneous membrane, the zona pellucida (fig. 3). In several cases this is completely surrounded or only partly covered by a thin layer of albumen laid down in several laminae. The zona can be easily distinguished from the albumen on account of its dense, homogeneous structure. In some eggs the zona is not so well recog-

nized, the outline of the egg being diffuse and not bounded by a definite line. The zona is 0.004 to 0.0008 mm. in thickness.

Beneath the zona pellucida is a layer, very light in color and with very fine radial striations, clearly seen under the oil-immersion lens. In most eggs this has a uniform width of about 0.0012 mm. Whether or not this is in any way homologous to the zona radiata I am not prepared to say.

The cytoplasm is very definitely marked off from the lighter 'zona radiata' just mentioned. In fact, in some specimens the line of demarcation is so definite as to amount almost to a membrane.

As seen in sections the cytoplasm of the egg may be divided into three more or less distinct regions (figs. 1 and 3). The first of these is a narrow homogeneous band of finely granular protoplasm, devoid of yolk granules or yolk vacuoles. The band is darker than other portions of the cytoplasm, except occasional islands of granular structure surrounding yolk vacuoles. The center of the egg consists of a similar, homogeneous cytoplasm, granular throughout, with only here and there a fat vacuole. The central area extends from the center about half-way to the circumference. Between the marginal and central homogeneous portions of the cytoplasm is a broad band of highly vacuolated, reticular protoplasm, very clearly seen, especially in the eggs of lot No. 76. The structure of this ring seems to be the most delicate of the entire egg, for in two or three specimens the cytoplasm is somewhat broken down at this place. The vacuoles exist, even in eggs fixed with osmic acid mixtures (fig. 1). Sections of such eggs show also a ring of fat spherules, large and small, scattered around the margin of the central yolk free area. The fat spherules are numerous and may occupy a large portion of the vacuolated zone (fig. 1). In the region of the polar body the granular cytoplasm may come to the surface; in other words, there is at this point a break in the vacuolated zone.

The first polar body and the egg nucleus are to be seen in every specimen sectioned. Neither has undergone any material change since the egg left the ovary. The polar body is very small, containing a mass of chromatin and a minimum of cytoplasm.

In one case the chromosomes of the polar body were arranged in the equatorial plate of a division spindle. The position of the polar body is, in the majority of cases, though by no means invariably, at one pole of the elliptical egg, and the egg chromosomes lie in the cytoplasm just within the surface of the egg, either exactly beneath the polar body, or, as is more often the case, to one side of it. In a considerable proportion of the eggs, the polar body is found at the equator of the egg, less often at various levels between the pole and the equator, a variation found to obtain also in ripe ovarian eggs.

In a few of the eggs, particularly those of batch No. 56, one very large fat vacuole (or black fat spherule if stained with osmic acid) occurs at one pole of the egg, usually opposite the polar body (fig. 4). Many eggs also show a peculiar body about the size of a large fat spherule. It is homogeneous in structure devoid of all granulation, takes a pink or lavender stain with iron-haematoxylin and is surrounded by a light band, as if it had shrunk away from the surrounding cytoplasm. Its position in the different eggs is variable. Similar bodies have been noticed in other mammalian eggs.

Aside from the position of the polar body there is no evidence of polarity in the tubal egg of the opossum, such as has been described for ovarian and unsegmented uterine eggs of Dasyurus. In only one case was there a preponderance of yolk granules at one pole as compared with corresponding yolk-free granular cytoplasm at the opposite or 'animal' pole.

Insemination of the ovum no doubt takes place in the oviduct; for after the egg has received the albumen layer and the shell, the latter forms an impenetrable barrier to the entrance of the spermatozoa. Direct observations, however, of normal in semination in marsupials is lacking. In this connection Hill ('10) says of the eggs of Dasyurus: "Apparently the ova are shed almost simultaneously, and they must pass with considerable rapidity down the tubes to the uteri where cleavage begins, for I have only once found a tubal ovum, and that one had evidently been retarded for some reason, and was polyspermic."[3] Selenka

[3] Loc. cit., pp. 22-23.

('87) states: "Der Eintritt der Spermatozoen in den Perivitellin-raum geschieht im oberen Theile des Oviducts, wie die Anwesen-heit derselben in den auf dieser Strecke vorgefundensen Eiern genügend beweist."[4] But it will be observed that the actual entrance of the spermatozoon was not seen by Selenka, nor does he anywhere mention the pronuclei.

The ovum becomes surrounded with an albumen layer and a shell on its descent down the oviduct (fig. 5, 3). The albumen is laid down in successive delicate lamellae, between which numerous spermatozoa are seen in some of the eggs; or epithelial cells from the oviduct may become included in the albumen. One case was noted in which the spherical mass of epithelial cells instead of an ovum became surrounded by albumen and shell. In another case an ovum and a mass of cells were both included, and in a third case, two eggs, each separately provided with some albumen, were enclosed together in a shell.

c. *Appearance of young uterine eggs*

The eggs of female No. 58 are, to all appearances, the youngest found in the uterus. They are all unfertilized but otherwise are normal and show little or no signs of disintegration. Since the egg protoplasm very rapidly breaks up into fragments of various sizes, we may assume that the eggs of this lot had just arrived in the uterus. The shell membrane is, moreover, only 0.001 mm. in thickness and is so delicate as to shrink into many folds which lie, in the fixed specimens, snugly against the sphere of coagulated albumen. The shell grows in thickness in the uterus, hence the thickness of the shell is roughly a criterion as to the age of the eggs. But this is true only in very general terms, for there is no uniformity of thickness of shell among different batches of eggs in the same cleavage state (see Summary of this paper). The eggs of various individuals may, of course, pass through the Fallopian tube at different rates, hence the eggs passing the more slowly would be provided with thicker shells on entering the uterus.

[4] Selenka ('87), p. 110.

The yolk in the eggs of No. 58 is distributed more evenly and in smaller granules than in tubal eggs described above, but the three regions may be made out. These eggs, if freed of albumen and shell, would be almost exact counterparts of the eggs just entering the Fallopian tube (Nos. 56 and 76). In the preparations the shrunken shells measure 0.25 x 0.22 mm. in diameter; the ovum proper, however, measures 0.125 x 0.092 mm.; about the same as the eggs of Nos. 56 and 76.

The polar body and egg chromosomes occupy the same relative positions as in the tubal ova. There is normally no 'perivitelline' space, as described and figured by Selenka. Sometimes, indeed, the zona pellucida cannot be disinguished, the protoplasm of the egg grading off, somewhat abruptly it is true, into the albumen layer, and in these cases no zona can be seen. Among some 230 preparations only rarely do I find any space between the egg and the albumen, hence I believe that the 'perivitelline space' of Selenka is an artefact due to shrinkage. In several eggs of batch No. 54 the protoplasm was slightly shrunken away from the zona, not the zona from the albumen. Yolk is eliminated in the 2- and the 4-celled stages, and lies between the blastomeres and the zona. After this the blastomeres become closely applied to the albumen layer, with the zona pellucida still apparent between the two in some cases.

When observed in the fresh state in the uterine liquid or in Ringer's solution the young eggs resemble markedly a half-cooked grain of tapioca or sago. The shell and albumen form a hyaline, almost transparent envelope with a white granular, rather opaque central core, which is the egg proper. The shell is turgid and well rounded out. As the fresh egg may measure through the shell 0.4 to 0.5 mm. (e.g., D text fig. 4, page 34) the albumen layer is seen to be much thicker than that of Dasyurus and the study of the small ovum in the center correspondingly more difficult (fig. 4). In the fixing fluid the albumen becomes so opaque that the egg cell can scarcely be seen, especially when the fixing fluid contains no osmic acid to differentiate the egg by the black stain of its yolk. On prolonged immersion of the egg in alcohol, the albumen clears up somewhat, so that the

ovum may again dimly be seen in the center. The concentric lamination of the albumen is apparent in the fresh state and in sections.

d. *The pronuclear stage*

The eggs of lots Nos. 54 and 52, among which specimens of the pronuclear stage are found, are apparently young eggs which have just entered the uterus. The shell of batch No. 54 is 0.001 mm. in thickness, that of No. 52, 0.0034 mm.

The pronuclei at first lie to one side of the egg and are surrounded by an almost yolk-free area of considerable size (fig. 4). To this extent the egg at this stage possesses an obvious polarity. Later, however, the yolk-free cytoplasm surrounding the pronuclei occupies approximately the center of the egg (fig. 6), and here the first cleavage spindle will form (figs. 7 and 8).

Egg No. 54 (2) d (fig. 4) was sectioned in such a manner that both pronuclei lie in the same section. The specimen appears normal in every way except for a third body situated between the pronuclei and in contact with one of them, as if extruded from it. This body has a structure similar to that of the pronuclei, and contains a little chromatin substance. Figure 6 is made from a section containing one pronucleus and the second pronucleus was inserted from its position in the sixth section beyond (sections 6 μ thick). In another case, No. 54 (2) a, the male pronucleus seems to be in the act of withdrawing from the surface as if the spermatozoon had just entered; for the pronucleus is top-shaped with the point at the extreme surface of the egg.

Neither the first nor the second polar body has been recognized with certainty in these eggs. This is perhaps due to the interference of yolk granules, not thoroughly bleached of osmic acid stain, and to the presence of numerous epithelial cells from the tuba, imbedded between the laminae of the albumen.

The eggs in the pronuclear stage do not exhibit any marked polarity aside from the temporary position of the pronuclei. Sometimes, it is true, the yolk is more or less concentrated in large granules toward the pole opposite the pronuclei; in other cases, as noted, the polar bodies are centrally located. An

obvious polarity is to be recognized in several eggs of batch No. 52 in which no chromatin is seen, but in other eggs of the same batch with similar absence of well-stained chromatic substance, the yolk is peripherally situated. Whether one study a given section through the center of the egg or the series of sections as a whole, no striking condition of polar differentiation into yolk mass and cytoplasm is encountered nor are minor polar differences in structure of the egg at all of uniform occurrence during the pronuclear stage.

From these facts, it is apparent that the pronuclei, at first eccentrically situated, soon migrate to the center, and the yolk maintains its peripheral distribution. This interpretation finds ample confirmation from a study of the next stage.

THE FIRST CLEAVAGE

a. *The first cleavage spindle*

The first cleavage spindle was observed in two specimens: No. 54 (4) b in metaphase (fig. 7) and No. 52 (3) a in early anaphase stage of division (fig. 8). The former shows a slight polarity in the distribution of yolk vacuoles, but the chromosomes are situated centrally. In the other specimen, there is no trace of polarity. The central area is perfectly yolk-free and is largely occupied by the spindle, only partly shown in figure 8. At the periphery there is a considerable number of fragments of yolk eliminated (not shown in the figure). At one side a new cell membrane appears to be forming just within the eliminated yolk-laden fragments.

The formation of the first cleavage spindle from the pronuclei was not observed, but sufficient material has been described to make it certain that the entire egg in the first cleavage divides approximately, if not exactly, into equal halves and that the yolk is not cast off in one large polar mass as in Dasyurus. That the yolk is given off at the entire periphery is shown by the stage just described and by the two-celled stage to be discussed in the next section.

b. *The two-celled stage*

Only three normal 2-celled stages, taken from two different females, Nos. 46 and 50, have been available. These specimens offer ample corroboration of the manner of cleavage and yolk elimination foreshadowed by the eggs of the preceding stage containing the first cleavage spindle.

The first cleavage plane divides the egg into approximately equal halves. While no quantitative difference is to be recognized, there seems to be a qualitative difference between the two blastomeres; for in one case the separation of one blastomere from its yolk has not yet been consummated (fig. 9). In other words, one blastomere is in advance of the other. This apparently insignificant difference may not be without meaning, for there is nothing in the subsequent history of the early cleavage out of harmony with the assumption that the daughter cells of one blastomere develop into the formative region, those of the other blastomere into the non-formative region of the blastocyst.

No polarity, either in the distribution of the yolk granules or in the position of the nuclei, can be recognized. In one section more yolk may appear at one pole of the cell; in the next section the other pole may contain the greater number of yolk vacuoles. This absence of polar differentiation is quite in harmony with the condition of the 4-celled stage presently to be described.

c. *The elimination of yolk*

The manner in which the yolk is given off is of importance. One cannot speak of 'extrusion' of yolk, for it is not cast out bodily as in Dasyurus; hence, the term 'elimination' is employed. The egg gets rid of surplus yolk by forming the new cell membranes of the two blastomeres at such a distance from the original surface of the egg as to leave a portion of the peripheral cytoplasm rich in yolk outside the blastomeres. This process is evident from a study of No. 50 (6) shown in figure 9. One blastomere has rid itself of yolk and has become rounded out. The other blastomere has not yet completed the cutting off of

the yolk, but the future surface of the blastomere is distinctly outlined by a marginal, yolk-free zone along which the membrane of the blastomere will form. In other words, one blastomere was caught in the act of eliminating its yolk. In the other specimen, No. 46 (1) shown in figure 10, both blastomeres have eliminated the yolk, but one has rounded off in advance of the other. After the cells have separated and become fully formed, the yolk remaining within them comes to have the same distribution in a submarginal region as in the unsegmented egg. This peripheral distribution is to be seen in blastomeres up to the 16-celled stage (fig. 23). The eliminated yolk and cytoplasmic substance, together with the layer of albumen, is later completely but slowly absorbed. Considerable yolk, in variable amounts, is retained even within the cells which form the walls of the younger blastocysts. But this yolk is also gradually absorbed until in late unilaminar blastocysts the fat granules are only occasionally observed.

It follows, then, from the absence of polar distribution of yolk in the opossum egg as compared with the egg of Dasyurus, that the yolk of the former is not given off as in the latter. In the opossum egg the yolk, being peripherally distributed, is given off peripherally; being concentrated in a dense mass at one pole in the egg of Dasyurus, the yolk is, in this case cast out at the pole when the two blastomeres form. The egg of the opossum is also much smaller (text fig. 4) and probably also proportionately less laden with yolk, for its egg measures on the average about one-half the diameter, that is, one-eighth the volume, of the egg of Dasyurus.

<center>THE 4-CELLED STAGE</center>

<center>a. *The orientation of the blastomeres*</center>

1) *Shifting of the blastomeres.* The orientation of the blastomeres in the 4-celled stage presents the chief point of divergence in the cleavage of Didelphys and Dasyurus, the former following the manner of Eutheria. The conclusions here presented are based upon abundant material secured from females just captured, as well as from cage animals.

It will be recalled that the four blastomeres of the correspond-
ing stage in Dasyurus are radially arranged and that each cell
shows distinctly an upper (formative) and a lower, yolk-laden
(non-formative) pole. The four cells, furthermore, maintain the
radial arrangement until the end of the third cleavages, which
are also meridional. In the 8-celled stage the blastomeres are
arranged in an open ring and each continues to exhibit a dis-
tinct polarity. The opossum egg, on the contrary, behaves at
this time like the Eutherian egg, for the blastomeres shift and
cleavage becomes indeterminate.

The second cleavage plane in the opossum is at right angles to
the first, so that lines joining the centers of blastomeres having
the same origin at first lie parallel. Several eggs show this
parallel arrangement more or less distinctly, especially 52 (3) d,
which has apparently just divided, for a membrane can be seen
to have formed between each pair of blastomeres and the two
cells of each pair are still in contact.

After division has been completed the cells assume the form
of an almost perfect sphere. No exception to this has been
seen in nineteen excellent preparations of this stage. The cells
lie free in the semi-liquid egg content. They may or may not
be in contact with each other and seldom touch the zona pellu-
cida, which in some specimens, can still be seen closely appressed
to the albumen layer. The four blastomeres do not show the
slightest trace of polarity, being spherical in shape and having
the nucleus usually in the center.

It is little wonder, then, that the blastomeres should shift
their relative positions, and this is exactly what happens. The
pairs of cells, at first parallel, shift until they lie at right angles,
like the balls of a pair of crossed dumb-bells. The preparations
show every gradation in the process of shifting. Drawings
from wax models of these eggs clearly illustrate these points
(fig. 1). The shifting of the eggs can also be seen by an inspec-
tion of figures 11 to 16, which represent three eggs, a separate
drawing being made for each pair of blastomeres. The fragment
of shell or the configuration of the albumen about the egg will
enable the reader to note the angle taken by lines passing through

the centers of the two pairs of blastomeres of any one egg. Some of the eggs of this batch were studied in alcohol and, after staining with Delafield's, also in xylol. To facilitate the study of eggs in toto, the shells were removed, when the arrangement of the two blastomeres could be plainly made out. That the pairs of blastomeres of these eggs lie at different levels is shown by the fact that it is impossible to orient the eggs in such a way that a single section may pass through all four of the cells. Usually only two cells are found in any one section (figs. 11 to 16); some-
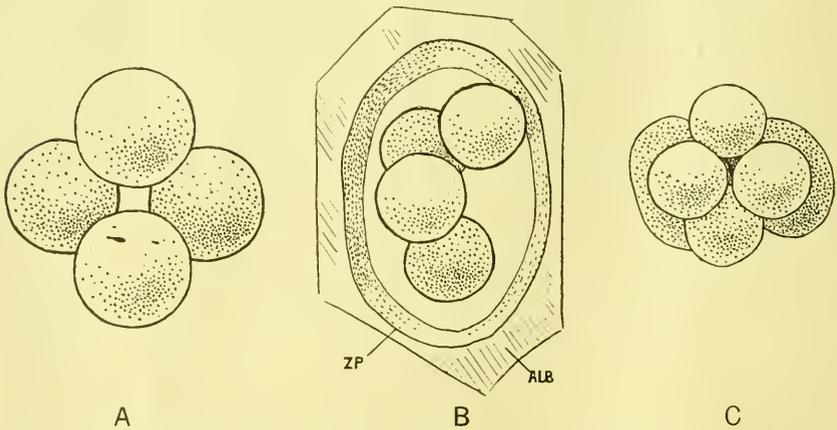

A   B   C

Fig. 1 Sketches made from wax models of 4- and 6-celled stages. *A*, No. 83 (2); *B*, No. 81 (6); *C*, No. 117 (4). In *B*, *ZP*, zona pellucida; *ALB*, albumen. The space between blastomeres and zona in *B* is occupied by yolk and coagulum (× 330).

times a third cell may also be cut by the section (figs. 17 and 18).

In the 6-celled and 7-celled stages (text figs. 1, *C* and 2, *A*) the position of the pairs of daughter cells bears out the process described above for the 4-celled stages.

It is thus seen that if any polarity could be attributed to the undivided opossum egg on account of the original position of pronuclei at one side, or of other facts presented above, no stretch of the imagination could find a trace of polarity in the 4-celled stage. The blastomeres appear to be identical and shift their position as in the Eutheria.

2) *Consequences of shifting of the blastomeres.* The shifting of
the blastomeres in the 4-celled stage results in an entirely differ-
ent type of cleavage from that obtaining in Dasyurus, in which
both qualitative and quantitative features are such as to enable
the observer to trace out the fate of the blastomeres. The cell
lineage thus determined is made more certain because the blasto-
meres of the 8-, 16- and 32-celled stages maintain a constant and
definite relation to each other. In the opossum egg the absence
of polar differentiation into large and small or light and dark
blastomeres increases the difficulty of determining the cell
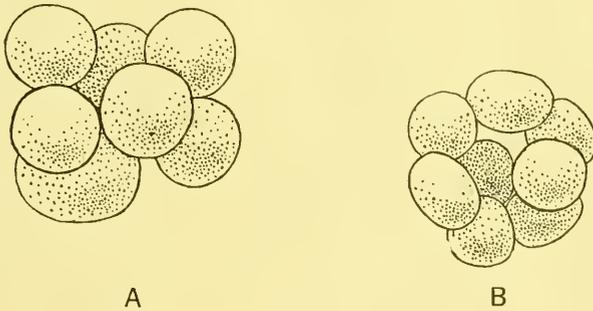lineage.

A   B

Fig. 2 Sketches from wax models: *A*, of 7-celled egg, No. 85 (7), and B, of
8-celled egg, No. 117 (10). Seven of the eight cells of *B* are in mitosis. Blasto-
cyst formation is already anticipated in these eggs ($\times$ 330).

In the 4-celled egg of the opossum in which the axes of the
respective pairs of daughter cells are at right angles, one pair is
clearly 'above' the other (text fig. 1, *A* and *B*). It is not im-
probable that the two pairs have different destinies. Certainly
the blastocyst of the opossum agrees with that of Dasyurus in
a polar differentiation with formative and non-formative regions.
In Dasyurus the differentiation of the two kinds of cells comes
in the fourth cleavage. In the opossum egg no differentiation
is apparent in the cleavage stages, but there must needs be a
potential difference in the cells of the two poles. If present in
the 16-celled egg of the opossum, as in the 16-celled egg of Dasy-
urus, the polar differences already exist in the 8-celled, the 4-
celled and the 2-celled egg. Hence, also, the opossum egg

possesses a potential, though concealed, polar differentiation into formative and non-formative cells. This I believe is the true interpretation. The subject will be pursued further below.

b. *Selenka's four-celled stage*

The normal 4-celled egg of the opossum as here described is totally at variance with the single 4-celled specimen seen by Selenka[5] as well as with the normal stage in Dasyurus. But I am fully convinced that Selenka's only 4-celled specimen was abnormal and in the process of disintegration. I have several times seen such eggs consisting of four radially arranged 'blastomeres' or egg fragments. A striking case is an egg of batch No. 112, in which the four cells seem an almost perfect replica of Selenka's specimen. At their upper ends the blastomeres fit together by their inner angular surfaces but their lower ends are spread apart. The cells are crowded to one pole of the egg, touching the zona with their upper ends, a condition to a certain extent true of the egg figured by Selenka. This egg was studied in alcohol and xylol in toto and was then perfectly oriented and imbedded in paraffin, and sectioned. The previous diagnosis of abnormality was sustained by a study of the sections. The nuclei of the cells were very abnormally placed and other signs of degeneration were apparent. All other eggs of this batch No. 112 were in various stages of disruption. I am perfectly convinced that Selenka's 4-celled stage is abnormal and does not represent the normal condition in the opossum, notwithstanding that it is comparable with the 4-celled egg of Dasyurus.

c. *The distribution of yolk*

The blastomeres of the 4-celled eggs of the entire collection are in the same stage of advancement; they have fully formed nuclei and no case of mitosis occurs among them. It would seem, therefore, that the blastomeres of the 2-celled stage divide practically at the same time. In all cases the relation of cells to yolk is the same.

[5] Loc. cit., p. 113; figures 4 and 5, plate 17.

The distribution of eliminated yolk is as would be expected, a priori, if one accept the interpretation of the 2-celled stage advanced above. If there were any doubt as to these views on the expulsion of the yolk in the 2-celled stage on account of the meager available material on which the conclusions were based, the explanation presented is fully justified by a study of the abundant material secured at the end of the second cleavage. The four blastomeres in all cases are almost completely surrounded by precipitated material, the 'yolk.' Rarely do the blastomeres touch each other and still more rarely are they in contact with the albumen layer or with the zona, in case the latter still persists.

The structure of the 'yolk' in the eggs of specimens Nos. 81, 83, and 54 is peculiar to these eggs (figs. 11 to 18) and was not met in any others, not even in the blastocysts furnished by No. 83, in addition to the 4-celled stages. It is a more or less homogeneous mixture of discharged cytoplasm, fat (yolk) and coagulum (protein of the egg sap)—all of these mixed together and precipitated by the fixing fluids into flocculent masses (fig. 18). There is often a more or less even distribution of small yolk granules (fig. 17) or fat vacuoles, but they are not as prominent as in other eggs. In other stages the eliminated material is usually massed in lumps, in which the black yolk globules or clear vacuoles are plainly seen; and in many eggs the coagulum appears as a thin precipitate separate from the other substances. Of the 4-celled eggs only egg No. 52 (3) d shows the usual appearance of the yolk; perhaps because of a different treatment of the preparation.

In less than half of the specimens at this stage one polar body was seen; in a few both polar bodies were found. The zona pellucida was observed in only two or three eggs. The blastomeres themselves have no cell membranes, excepting again No. 52 (3) d. The cells are usually well marked but there is no sharp, clear-cut line or membrane (figs. 17 and 18).

The blastomeres of the different eggs vary considerably in size, measuring from 0.043 to 0.056 mm. But this variation is not due to differences in size of eggs, that is, of volume enclosed

by the zona, for a large egg may contain small blastomeres. It is due rather to differences in quantity of yolk and cytoplasm eliminated in the previous stages; the more yolk thrown off the smaller the blastomeres. The end result is, however, the same, for the yolk is eventually absorbed and used as nutriment, whether it remains within the blastomeres or not. The yolk remaining within the blastomeres is arranged peripherally, as in previous stages, and indeed in the 8- and 16-celled ova as well.

The peculiarities of the first two cleavage stages of the opossum egg and their interpretation as presented above are fully confirmed by the succeeding cleavage stages now to the discussed.

THE 8- TO 16-CELLED STAGES

a. *Third and fourth cleavages*

1) *General statement.* Eggs of five to eighteen cells were furnished by specimens Nos. 85 and 117; these two batches included five 8-celled and eight 16-celled eggs. Every other number of blastomeres from five to eighteen inclusive was represented in different specimens. The eggs of No. 85 are to be considered perfectly normal, for the female furnishing them was fresh from the field and the eggs are of normal size (figs. 19–24). Yet the eggs of batch No. 117 appear normal in every respect except as to size (*A* and *B* text fig. 2). The reduction in size is to be expected in view of the fact that a single ovary of female No. 117 discharged 43 or more eggs at the second oestrus period, having one month previously discharged about twenty. The measurements of several eggs of both batches (measured from sections) are given in the following table:

TABLE I

		mm.			mm.
85 (7)	7-celled	0.124 x 0.095	117 (4)	6-celled	0.103 x 0.101
85 (18b)	12-celled	0.11 x 0.102	117 (10)c	10-celled	0.087 x 0.083
85 (3)	9-celled	0.13 x 0.11	117 (10)b	8-celled	0.093 x 0.084
85 (11)	16-celled	0.13 x 0.117	117 (11)a	16-celled	0.087 x 0.085

A study of this abundant material makes it certain that the third and fourth cleavages take place in the opossum ovum in a manner quite different from that shown by Hill to hold for the egg of Dasyurus. In that form the fourth cleavage is for the first time horizontal and divides the single ring of eight conical cells into an upper ring of small, relatively yolk-free cells and a lower ring of larger, yolk-rich and more darkly staining cells. The former are said to be destined to produce the formative portions of the blastocyst. Thus a single section of the 16-celled egg may pass through all of the eight blastomeres of a given ring (text fig. 4, *E*). This description does not hold in the slightest degree for the opossum egg in the corresponding stage.

The blastomeres of the 4-celled opossum egg having shifted about at right angles as described above, one would expect the planes of the third cleavage also to pass irregularly and at various angles through the four blastomeres. This is the case. The shifting of the blastomeres continues as cleavage progresses. The cells migrate more and more to the periphery and spread about irregularly into the form of a hollow sphere. When the 16-celled stage has been reached, or even before, all semblance of orderliness seems to have vanished.

In another respect the opossum egg of sixteen cells differs from the corresponding stage of Dasyurus (compare *E* and *F*, text fig. 4). In the latter species the albumen layer is entirely absorbed and the progeny of the sixteen blastomeres, as these multiply, come at once to lie against the shell. In the 16-celled stage of the opossum, however, there has been as yet no perceptible absorption of albumen, the layer of which is enormous as compared with that in the egg of Dasyurus. The albumen of the opossum egg is not exhausted until the bilaminar blastocyst is some 1.4 mm. in diameter and the formation of the mesoderm and the primitive streak is initiated (Selenka).

2) *Cleavage indeterminate.* After the opossum egg has passed the 4-celled stage the planes of cleavage of any two dividing cells are never parallel; that is, the axes of the division spindles in any given stage point different directions. Already in the 6- or 7-celled egg (text figs. 1 and 2), the continued shifting may

be noted from a study of wax models of such eggs. In models of eggs from the 6- to the 16-celled stages the blastomeres are grouped into pairs of common origin. Thus a 6-celled egg has two large cells and two pairs of small cells, that is, $2 + (2 \times 2) = 6$. Likewise, a 10-celled egg has six large and four small cells, or $6 + (2 \times 2) = 10$. If lines be drawn connecting the centers of the cells of each pair, scarcely any two lines are parallel (text fig. 3).

One need not rely upon wax models, however, to note that the cleavage planes are no longer parallel. Several eggs were fixed at the time two or more of their blastomeres were in mitosis and

A   B
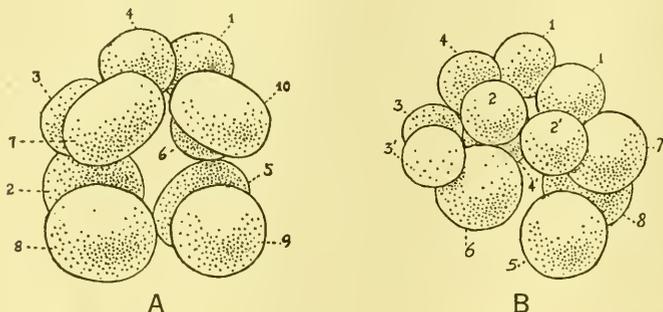
Fig. 3 Sketches of wax models during fourth cleavage. *A*, 10-celled egg No. 85 (18) a; cells 1 and 6 and 3 and 4 are pairs of cells of common origin; cells 9 and 10 are in mitosis. *B*, 12-celled egg No. 85 (18) b; the four pairs of smaller daughter cells are easily recognized in the figure; cells 5 and 6 are in mitosis (fig. 21) ($\times$ 330).

from the direction of the spindles the angle of the cleavage planes may be determined. This is strikingly true of three eggs containing 8, 10 and 12 cells respectively (text figs. 2 *B*, and 3 *A* and *B*). In the 8-celled egg (text fig. 2 *B*) two cells are in the telophase stage of division, the nuclei of the daughter cells undergoing reconstruction; five cells are in metaphase or beginning anaphase with the chromosome-loops clearly seen at or near the equator of the cells; one cell is in the resting condition. The directions of the spindles obey no apparent rule except that these are tangential to the surface of the egg, as the mitotic figures show.

The 10- and 12-celled stages are also instructive in this connection. In the 10-celled egg (text fig. 3 *A*) two blastomeres have just divided and two others show mitotic figures (late anaphase). Blastomeres 3 and 4 and 1 and 6 make two pairs respectively; 9 and 10 are in mitosis, with their spindles oriented at right angles to each other. Cell 7 is elongated like cell 10, as if soon to divide. All of these divisions are tangential to the surface, but besides this no order in arrangement of cleavage spindles can be noted. The same is true of the 12-celled egg (text fig. 3 *B*). Eight small blastomeres are irregularly massed to one side or 'pole' of the egg and are definitely arranged in pairs. Four larger cells, also irregularly placed, are found at the other side. Two of the larger cells are in mitosis (fig. 21) and the axes of their spindles are almost at right angles and nearly if not quite tangential to the surface.

The cases just described leave no doubt, therefore, of the fundamental differences between the fourth cleavage of Dasyurus and of the opossum. In the latter the 16-celled stage is established by an indeterminate cleavage of the cells, the division planes passing through the cells in no apparent order except that the spindles are tangentially arranged with respect to the more or less imperfect sphere made by the blastomeres.

The indeterminate type of cleavage as just described recalls a similar process obtaining in the Eutheria. The similarity between the early cleavages of the opossum and the higher mammals is further illustrated by the disparity in the time that cleavage of the various blastomeres takes place; for eggs of every number of cells, from five to eighteen, were found along with the 8- and 16-celled eggs. These occurred, however, in larger numbers than other cleavage stages.

3) *The 16-celled egg practically a blastocyst.* The blastomeres of the 16-celled stage are arranged about a cleavage cavity of considerable size (figs. 22 to 24). They are irregularly scattered in the form of a sphere with gaps of various sizes between most of the blastomeres, for but few of the cells are in contact with each other. They are in contact rather with the albumen layer, against which they tend to flatten out. In Dasyurus, on the con-

4
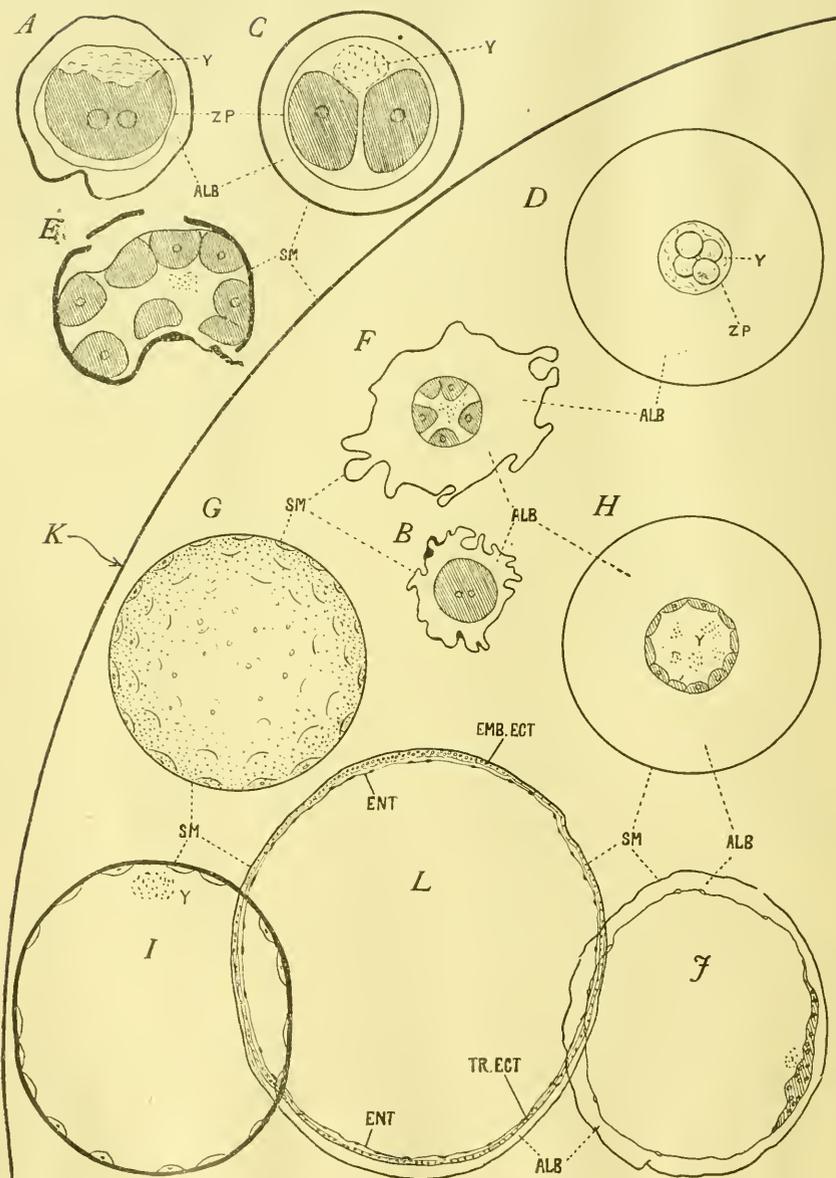
trary, the two superimposed rings maintain a position more or less parallel with the equator, so that a given section through the egg in the plane of the equator would pass through all of the eight cells of either ring (text fig. 4 E). A section through a 16-celled egg of the opossum (figs. 22–24) never passes through the centers of more than four or five cells. Such a section of the 16-celled opossum egg is not unlike a section through the 16-celled stage of a Eutherian mammal (fig. 22).[6] The early arrangement of the cells of the opossum egg into a hollow sphere makes possible the early formation of the completed blastocyst. The blastomeres in the eggs of Dasyurus, which are arranged in rings near the equator, must proliferate toward their respective poles. The blastocyst is completed when the cells have met and closed the gap at either pole. In the opossum egg the blastocyst is completed when the scattered gaps seen in the wall of the 16-celled eggs have been filled by a further division and a flattening of the cells. This process may be completed in this way in eggs having as few as thirty cells.

The blastocyst formation, however, is anticipated in the 8-celled stage or indeed even before this time. For in 5-, 6- and

Fig. 4 Diagrams to show comparative size and structure of the eggs of Dasyurus and Didelphys ($\times$ 75). A, C, E, G, I and K, eggs of Dasyurus (all except K after Hill); B, D, F, H, J and L, eggs of Didelphys (original). ALB, albumen; $EMB.ECT$, embryonic ectoderm; $TR.ECT$, trophoblastic ectoderm; SM, Shell membrane; Y, yolk; ZP, zona pellucida.

A and B, pronuclear stage; the ovum of the opossum is seen to be much the smaller; the yolk mass (Y) is seen at one pole of the egg of Dasyurus. By comparing C, a fresh 2-celled egg of Dasyurus, with D, a fresh 4-celled egg of Didelphys, one can readily note the relative size of entire egg and of ovum proper and the relative thickness of albumen and shell membrane of the two species. Note extruded yolk in C and D. Compare also E and F. E represents a section taken through an entire 'ring' of the 16-celled Dasyurus egg; no such ringed arrangement occurs in the opossum egg of 16 cells (F). G (surface view) and H (section) are blastocysts of equal size, but in the former the cells lie against the shell membrane, whereas in the latter the blastocyst itself is only a little larger than the unsegmented ovum. J, opossum blastocyst at time of entoderm formation; K, are of Dasyurus blastocyst in corresponding stage, after long period of growth, which does not occur in Didelphys. I, section of blastocyst of Dasyurus early in the period of growth, the period of 'no differentiation' (Cf. 1 with G and J). L, section of complete bilaminar blastocyst of Didelphys.

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[6] Cf., for example, Assheton ('98), figure 6, plate 15.
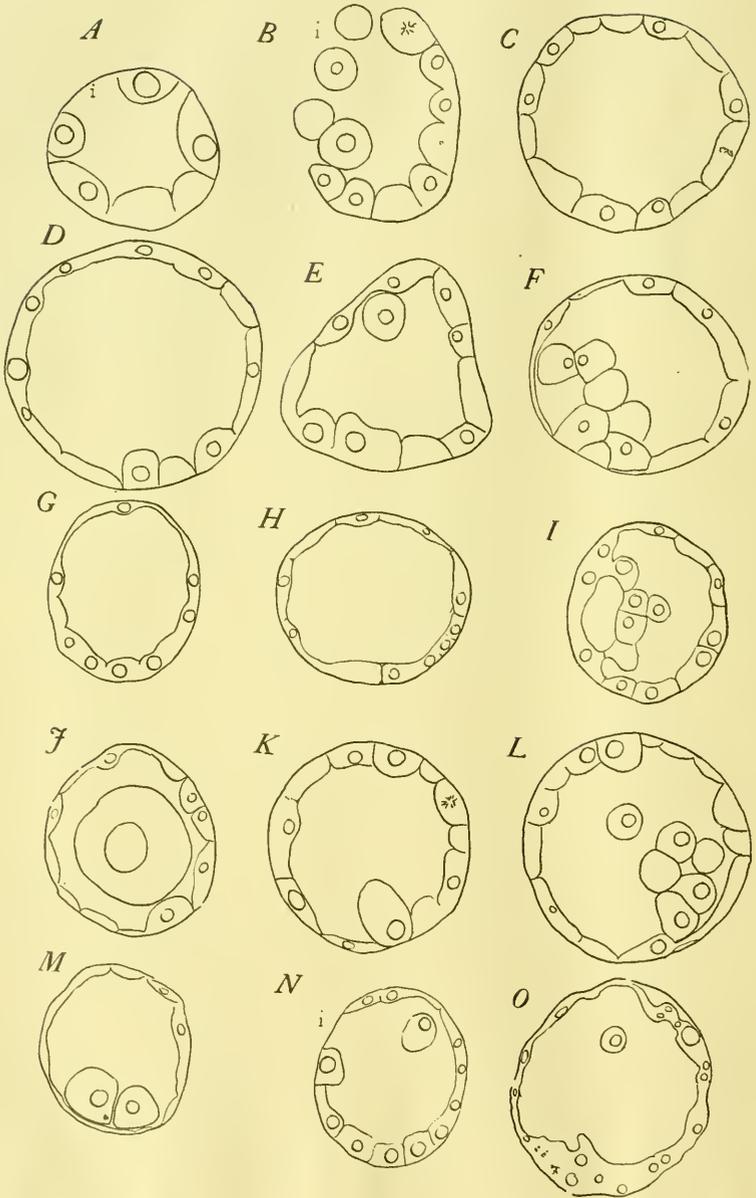
7-celled stages the tendency of the cells to migrate toward the periphery and to become closely appressed to the albumen layer is very apparent. One 12-celled egg is an almost fully-formed blastocyst (text fig. 5 A); the cells are very flat and closely applied to the albumen, precisely as in eggs of more than double the number of cells. On the other hand, in a number of 8- to 16-celled eggs, not all of the blastomeres touch the albumen layer and in these cases they maintain their spherical shape (fig. 21). Attention should be called at this point to a 16-celled egg, 85 (12), of which a model was made. This egg is unique for specimens of this batch in that it contains an included cell some distance removed from the peripheral cells making up the wall of the 'blastocyst' and so situated as to make it improbable that it could ever have attained a place in the wall of the blastocyst. This egg will be referred to again.

b. *The eliminated yolk*

The processes in the development of the opossum egg up to the 16-celled stage described above fully answer the question propounded by Selenka as to the manner in which the yolk reaches the cavity of the blastocyst. To quote:

Auf welche Weise die in Figure 8, 10 and 11 mit i bezeichneten Dotter-ballen in die Furchungshöhle gelangen, weiss ich nicht zu sagen; in d·n ersten beiden Stadien der Furchung fehlen sie ganz, ebenso sach

Fig. 5 Sections through early unilaminar blastocysts ($\times$ 250). i, gap in wall of blastocyst.

A, early attempt at blastocyst formation in 12-celled egg No. 144 (1) b. B, 31-celled egg, No. 18 (18), with only half of blastocyst formed normally. C, No. 88 (1), the only blastocyst here figured which is entirely devoid of included cells. D, No. 50 (4), 50-celled blastocyst with almost uniform wall; this specimen has several included cells, not shown in this section. E, No. 88 (2), shows polar differentiation in its greater thickness of cells at one pole as compared with cells at the opposite pole (Cf. G, O and others). F, No. 88 (14), showing excessive proliferation from cells in wall of blastocyst (Cf. I and L). G and N, two sections of No. 88 (4). H and I, two sections of No. 88 (7). J and M, two sections from opposite poles of No. 88 (6); note large included blastomere in J. K and L, two sections of No. 88 (16); note large cell in wall of K, and included cells in L. O, more advanced blastocyst, No. 144 (1) a; transition between the series shown in this figure and those shown in figure 6.

in den älteren Gastrulastadien; man wird daher zu der Annahme
gedrängt, dass diese Dotterballen von den Furchungszellen ausge-
stossen, bald aber wieder resobirt werden.[7]

Selenka was thus of the opinion that the yolk is given off in the
8-celled stage and not earlier.

It has been shown above that in the 4-celled stage of the
opossum egg the yolk practically surrounds the blastomeres.
In the next division the blastomeres exhibit a centrifugal tend-
ency and sooner or later come to lie closely appressed to the
albumen layer. Hence the eliminated yolk comes to occupy a
central position until absorbed. By what physiological activities
the blastomeres are forced against the albumen I am not pre-
pared to say. The fact is they do pass peripherally and occupy
a point of vantage where they may best absorb the nutriment
from the albumen and the included yolk as well.

The distribution of yolk remaining within the blastomeres in
eggs from five to sixteen cells occurs as in preceding stages,
namely in a peripheral belt (figs. 19 and 23). The relative
quantity of yolk within and without the cells is again a variable
one. Where the blastomeres touch the albumen layer there is
often less yolk or even none at all, the yolk granules or vacuoles
being concentrated at the surface toward the cleavage cavity
(fig. 23). Preparation of eggs fixed in Bouin's fluid show large
vacuoles at the rounded free side of the cell. Sometimes these
vacuoles coalesce into irregular spaces as if the cells broke down
at this point under the action of fixatives, which recalls the
behavior of the delicate deutoplasmic portion of the unseg-
mented egg of Dasyurus.[8]

c. *Absence of polar differentiation*

Although there is a shifting of blastomeres in the egg of the
opossum after the second cleavage, the daughter cells of either
of the first two blastomeres are, no doubt, to be found in one or
the other hemisphere of the 16-celled stage (text figs. 1 to 3). In
models of certain eggs containing 6, 7, 8, 10, 12, and 16 cells

[7] Loc. cit., p. 115.
[8] Hill, p. 12.

respectively, the blastomeres are very clearly arranged in pairs, the two daughter cells of any given division remaining close together for a time, their common origin being judged by the juxtaposition. The blastomeres of any given stage do not shift about sufficiently so as to destroy the orientation here implied, namely the identical lineage of all of the cells in either hemisphere of the egg.

This being true, a thorough search was made for structural evidences of polarity: differences in size, staining qualities and yolk content of cells. None were discovered. The cells in section usually appear of uniform size, except, of course, in eggs in which certain blastomeres have divided in advance of others (text figs. 1 to 3). The differences that may occasionally be noted in 8- or 16-celled eggs are sporadic and accidental and are not to be correlated with polar differentiation, as both sections and models of these eggs show. Neither were the differences in staining qualities or yolk content of the cells at all regular in any given egg.

Especially favorable specimens for testing the assumption of polar differentiation among the constituent cells are three eggs of 6, 10 and 12 cells respectively, already referred to in another connection. The 6-celled egg (fig. 1 C) has four small cells at one 'pole' and two large cells at the other $[2 + (2 \times 2) = 6]$. The 10-celled egg (text fig. 3 A) has two pairs of small cells and two larger cells in one rather clearly marked 'upper' ring and four large cells in the 'lower' ring $[6 + (2 \times 2) = 10]$. The 12-celled egg (text fig. 3 B) is similar to the 10-celled egg just described but has four cells divided into pairs $[4 + (4 \times 2) = 12]$. The smaller cells are concentrated at one pole. From models made of these eggs this orientation is clearly seen.

Fortunately, in these three eggs the sections were taken vertically, that is through both the divided cells at one pole and the undivided cells at the other. Here, then, if anywhere, are specimens exhibiting a polarity, slight though this be, in the retardation of cells division at one pole of the egg. If a comparison with the egg of Dasyurus in this regard be valid, the more rapidly dividing cells are formative, the others non-formative.

A careful study and painstaking comparison of the cells at one 'pole' with those of the opposite pole led to no discovery whatever of qualitative differentiation among the cells. Though the differences in the rate of division of the cells may have some significance they are certainly not correlated with structural differences.

That the 16-celled stage of the opossum, in common with the corresponding stage in Dasyurus, possesses a polarity, seems highly probable. But, unlike the egg of Dasyurus, the polarity of the opossum egg is only potentially present and is not reflected in structural differences. Polar differentiation begins in the blastocyst of about 40 cells, that is, soon after the fifth cleavage, when the blastocyst is just completed. As the polarity of the egg becomes manifested so soon, the assumption that polarity is also present, though concealed, in the 16-celled stage, is not at all unreasonable. If, therefore, the egg of the opossum in the 16-celled stage possess a potential polarity, even though this be not reflected morphologically, such polarity must needs be established before the 8-celled stage is reached—indeed, upon the completion of the first cleavage. For, having arrived at the 8-celled stage, the blastomeres are so shifted and arranged around the surface of a sphere that the 'fourth cleavage plane' could not possibly divide "the blastomeres of the 8-celled stage to form two superimposed rings," as in Dasyurus. Again, supposing that the fourth cleavage does divide each of the eight scattered blastomeres qualitatively into a formative and non-formative cells, the formative blastomeres resulting from the division of the four cells at the lower pole would have to migrate to the upper pole and the non-formative halves of the divided blastomeres in the upper portion of the egg would have to migrate to the lower pole—a process not to be seriously considered in the face of the facts. Again, the qualitative differences among the upper and the lower cells must be present as early as the 2-celled stage, if present in the 16-celled stage, for the study of eggs of 4, 6, 7, 8, 10 and 12 cells shows clearly, as noted above, that the cells of either pole of the 16-celled egg are lineal descendants of one pair of blastomeres of the 4-celled egg. The

whole matter may be summarized by the statement that cleavage proceeds in the opossum from the 1-celled to the 16-celled stage superficially somewhat as in the Eutheria and without evident polar differentiation, but that potentially the cells at the poles differ, those of one hemisphere being destined to become the formative, those of the other the non-formative region of the early blastocyst.

PART II. THE FORMATION OF THE BLASTOCYST

THE EARLY UNILAMINAR BLASTOCYST

a. *The just completed blastocyst*

1) *Formation of the blastocyst.* In the preceding section it was seen that the formation of the blastocyst was plainly fore-shadowed in the 8- to 16-celled stages. Early in cleavage the cells migrate toward the albumen layer, to which they soon become closely appressed. The stage at which the cells arrive and begin to flatten out beneath the zona varies in different eggs. The process may begin in the 5-, 6- or 7-celled stage (fig. 19) or it may be delayed beyond the 16-celled stage. In one 31-celled egg (text fig. 5 *B*), for example, the blastomeres of only one hemisphere have flattened out and completed this half of the blastocyst; the cells of the other half are rounded and still have gaps between them. In most cases of 15- to 18-celled eggs, however, the blastomeres have definitely established a contact with the albumen and this relation they do not change until, in the large bilaminar blastocysts, the albumen is all absorbed. Hence in blastocysts of the opossum there is no 'peritivelline space' nor are the cells at all normally rounded off peripherally in the manner figured by Selenka. The early blastocysts described by him are no doubt damaged specimens in which the cells have shrunken away from the albumen layer.

Since, therefore, the blastomeres of the 16-celled egg are already arranged in a hollow sphere, the blastocyst is completed at a much earlier stage than that of Dasyurus. Of nearly four dozen specimens of young blastocysts, each approximating 50 cells, all have completed walls except five (text fig. 5 *N*). The blastocyst wall is indeed complete in some specimens containing from 30 to 40 cells.

In Dasyurus, on the contrary, blastocysts of 89, 93, 121, 128 and 130 cells are still incomplete, the last being on the point of closing at the poles. The difference between the two forms is due to differences existing between the respective 16-celled stages. In the Australian species the two rings of cells, equatorially placed, must fill the space from the equator to the poles by sheer force of crowding, as they multiply and spread. As they proliferate, the cells are held in the form of a sphere by the enveloping shell. In the opossum egg the cells are already distributed over the sphere in the 16-celled stage; hence with one more division and a little flattening of the cells the blastocyst wall is completed. At this stage the diameter of the eggs of Didelphys and Dasyurus is about the same, 0.45 mm. But this measures practically the diameter of the Dasyurine blastocyst, the cells of which are flattened out under the shell of the egg. In the opossum the thick albumen layer, hardly yet attacked by the metabolic activity of the cells, intervenes between the small blastocyst (0.12–0.15 mm. in diameter) and the shell membrane (*G* and *H*, text fig. 4).

2) *First appearance of polarity.* Selenka describes and figures two early unilaminar blastocysts of 42 and 68 cells respectively. Both exhibit an obvious polarity in that they consist of large, yolk-laden cells at the lower pole, and these taper off gradually to small, somewhat elongated, flatter and smaller cells at the upper pole. An inspection of figure 5 discloses undoubted evidence of polarity in the specimens of my collection. A polar differentiation is then apparent for the first time in the history of the egg. So far as I have been able to discover, however, the difference between the cells at the two poles of the blastocysts is one of thickness only, there being no difference in yolk content or staining qualities of the cells.

The differences in thickness of cells is sometimes considerable (text fig. 5 *E-H*), sometimes hardly noticeable (text fig. 5 *D*) and in some cases the wall appears practically uniform throughout (text fig. 5 *C*). The shape of the section, whether circular or elliptical, is not correlated with appearance of polarity. Orientation was not feasible in imbedding the specimens, since the

thickness of the albumen layer made it impossible to distinguish the thick from the thin-walled portion of the blastocyst. Hence, some eggs were sectioned equatorially, others meridionally, and still others obliquely. Measurements of cells through the series, however, indicated that in some of the blastocysts with about 50 cells a polar differentiation had not yet taken place. It must be assumed, therefore, that the greater flattening of the cells at one pole as compared with those at the other is delayed in some cases. In general however, the polar differentiation may be said first to manifest itself at about the 50-cell stage or almost immediately upon the completion of the blastocyst wall.

Occasionally one or more large cells occur at one pole of the egg (text fig. 5 K). Figure 25 is a blastocysts consisting of 52 cells. The large cell in the section is one of four similar cells in juxtaposition at this pole. Perhaps these represent a somewhat retarded generation of blastomeres. The case brings to mind such a cell in an advanced blastocyst already in process of forming the entoderm (figs. 37 and 38). Occasionally these larger cells situated in the wall are darker and have their long axes radially arranged (text fig. 5 K). Sometimes they are found in process of division.

In the opossum egg there is no polarity due to the presence of a yolk mass at the 'formative' pole, as in Dasyurus, nor to the presence of included cells at the 'entodermal' poles, as Selenka would have it. In a few cases, however, where there is a great attenuation of the cells at one pole of these early blastocysts, such thinning away may be due to the presence of large numbers of included cells. The matter of included cells will be discussed below.

3) *Inclusion within the blastocyst cavity.* There may always be distinguished within the blastocyst cavity such inclusions as were mentioned in connection with the 16-celled stage (figs. 25 and 26). Masses of coagulum (precipitated proteins of the blastocystic fluid) are seen in one part or the other of the cavity. Yolk granules are scattered in varying quantity throughout the cavity and are nearly always surrounded with cytoplasmic fragments. Most of these are the result of yolk elimination from

the 2-celled egg, as previously described. Their position within
the cavity of the blastocyst is due to the migration of the cells
to the albumen layer some time after the 4-celled stage is passed.

Besides these inclusions, the blastocyst cavity usually con-
tains entire cells as well as cell fragments bearing chromatin
matter (text fig. 5). Selenka also described and figured an
included cell in his 42 and 68-celled stages and mentions an
included cell in his much damaged 20-celled egg. Such cells
are found in the cavities of thirty-nine out of forty-four blasto-
cysts in my possession.

The cellular material mentioned in the preceding section comes
to lie in the cavity of the blastocyst in two ways: first, by failure
of cells in about the 16-celled. stage to reach the periphery and
second by proliferation from the blastocyst wall. The first
method seems highly probable, a priori, and is strongly sug-
gested by the presence of an included cell in the 16-celled egg,
specimen 85 (12), cited above. The model made of this egg
shows that the included cell had little chance of finding its
place in the blastocyst wall. The included cells of the 50-celled
stages are, moreover, in some cases fragmentary and show signs
of disintegration.

The second method suggested is apparent from an inspection
of certain blastocysts shown in text figure 5 (*F, I, L*). There
are four types of cells in these blastocysts: 1) the ordinary flat
to cubical cells definitely located in the blastocyst wall; 2) one
or more much larger cells, often columnar in shape, also situated
in the blastocyst wall and frequently darkly stained; these cells
sometimes jut out far into the cleavage cavity (text fig. 5 *K*);
3) included cells connected with the cells of the blastocyst,
clearly cases of proliferations from the cells of class 2; these
proliferated cells in several cases make a complete bridge across
a sector of the blastocyst (text fig. 5 *F*); 4) ordinary included
cells floating in the cavity singly or connected with other cells
as if just divided. Since the majority of these early blastocysts
are absolutely normal, the included cells doubtless have no
special significance. A study of blastocysts in the next stage
indicates that the included cells do not take part in the formation

of the entoderm, as suggested by Selenka. To suppose that the cells in various parts of the blastocyst cavity migrate to the formative pole of the egg, attach and proliferate as definitive entoderm of the blastocyst is asking too much of them; nor does this presumption accord with facts observed in studying the later stages.

It will be recalled that Selenka ('87) observed included cells in the 20-celled egg and in the two young blastocysts studied by him. Inasmuch as Hill ('10) criticised these stages as abnormal, and as the matter is one of importance, it will now be discussed in detail.

b. *Cells included in the blastocyst cavity*

Of the included cells above mentioned Hill says:

Whilst the 42- and 68-celled blastocysts described by Selenka may be regarded as normal so far as the occurrence of polar openings and the constitution of their wall are concerned, I hold them to be abnormal in respect of the presence in each of a single large yolk-laden cell, regarded by Selenka as entodermal in significance. It is well to point out that Selenka was not able actually to determine the fate of this cell; he merely presumed that it took part in the formation of the definitive entoderm. No such cell occurs in normal blastocysts of Dasyurus at any stage of development, and in my opinion Selenka's 'urentoderm-zelle' is no other than a retarded and displaced blastomere. . . . . I am strengthened in this interpretation by the occurrence in an abnormal blastocyst of Dasyurus of just such a large cell as that observed by Selenka. . . . . This cell corresponds in its size and cytoplasmic characters with a non-formative blastomere of about the 16-celled stage, and I regard it simply as a blastomere which has failed to undergo normal division.[9]

In two other incomplete blastocysts of less than 0.4 mm. diameter Hill found abnormal included cells; but in later blastocysts 0.5 to 0.8 mm. in diameter, taken from three different females, he often finds included cells or fragments thereof. To quote again:

In blastocysts of this stage of growth a variable number of small spherical cells or cell-fragments are frequently met with in the blastocyst cavity, usually lying in contact with the inner aspect of the cellular wall. In some blastocysts such structures are absent, in others

[9] Loc. cit., p. 42.

one or two may be present, in yet others numbers of them may occur. They may be definitely nucleated, but this is exceptional; more usually they contain one or more deeply staining granules (of chromatin?), or are devoid of such. They are of no morphological importance, and I think there can be no doubt that they represent cells or fragments of cells which have been separated off from the cellular wall during the process of active growth. They are of common occurrence in later blastocysts, and it is possible the so-called 'yolk-balls' observed by Selenka in Didelphys are of the same nature.[10]

It is thus seen that Hill does not consider his later blastocysts abnormal, no doubt because of the large proportion of specimens containing included cells.

Again, in later full-grown bilaminar blastocysts Hill finds islands of deep-staining cells, both in the formative and the nonformative areas, in contact with the blastocyst wall. These cells have large nuclei and may be actively proliferating or, on the other hand, they may be in process of degeneration. He considers them "of no morphological importance and (they) are destined sooner or later to degenerate." I have not seen cells like these last described, but Selenka mentions similar ectodermal growths in opossum vesicles containing embryos with three somites.

It is not amiss at this point to recall that cell fragments have been reported in normal cleavage stages of Eutherian embryos, e.g. by Assheton ('98) in the sheep. In the 8- to 17-celled stages of the sheep ovum the segmentation cavity "always contains spherical or otherwise shaped masses of substance in every way similar to the cytoplasm of the segments." In three specimens chromatin spots were recognized, in others chromatin-like granules. Of these Assheton says: "I cannot give any explanation of the fragments of cells seen within the segmentation cavity, nor of the origin of the nucleus-like body."[11]

These cases of included cells or cell fragments within the cleavage cavity as described by Selenka, Hill and Assheton for Didelphys, Dasyurus and Ovis respectively, are cited as probably illustrating cases of such inclusions in normal embryos.

[10] Loc. cit., p. 46.
[11] Loc. cit., p. 212.

In the forty-four preparations of young opossum blastocysts in my possession, taken from four different females, only five do not show clear cases of included cells with nuclei and in three of these cases I am in doubt as to whether or not chromatin fragments are present. If, therefore, cell-inclusion constitute an abnormality, my forty-four blastocysts are only 10 per cent or less normal. This I am certain is not the case.

There is, of course, every gradation in the volume of included material: lipoid, cytoplasmic and chromatic. There may be only a few fat grains and lumps of cytoplasm (fig. 25 and text fig. 5 *D*) or a single perfect cell in addition to these amorphous masses (fig. 26). Other eggs have two, three or a dozen small cells, or, as many or more large cells which may finally almost completely fill the segmentation cavity (fig. 5). One egg, indeed, is practically a solid morula, the cavity being all but obliterated by cell masses. But the included cells are fragmenting and the whole structure has the appearance of degeneration. This and other abnormal eggs are listed as such and are not among the forty-four on which the description of this stage is based. All of these forty-four I am convinced are normal and they are among the best fixed and stained preparations in my possession.

For three reasons, therefore, the conclusion is fully justified that blastocysts containing a limited number of cells within their cavities are perfectly normal, but that these cells have no morphological significance: 1) They are of undoubted occurrence in thirty-nine out of forty-four blastocysts of approximately the 50-celled stage, which are normal and the walls of which do not differ essentially from blastocysts apparently free of included nuclear material. The three cases of similar inclusions of cells observed by Selenka should also be noted in this connection. 2) Included cells without morphological significance have been observed in cleavage stages in other mammals. 3) Later stages of Didelphys and Dasyurus, apparently normal in every respect, show fragments of cells not entirely digested or absorbed.

To recapitulate, the blastocyst of the opossum, formed in the 16-celled stage, is completed soon after the fifth cleavage or at about the 40-celled stage. At this time or a little later a polar

differentiation may be made out for the first time in the development of the egg. This difference in the cells of the two poles is merely quantitative, so far as may be observed from a study of the specimens at this stage alone. The only evidence of polarity at this stage lies in the thinning of the cells at one pole of the egg. The question as to which of the poles is formative and which non-formative will be discussed in the next section.

THE FORMATION OF THE ENTODERM

a. *Entoderm formation as conjectured by Selenka*

The method of entoderm formation in the opossum natually interested Selenka, but he lacked the requisite stages to determine the matter. It has been shown above that the included cell in Selenka's 42- and 68-celled stages, celled by him 'Urentodermzelle,' has no morphological significance. His surmise, moreover, that the 8-celled stage is made up of four ectodermal and four entodermal cells is a mere conjecture, based solely on analogy. Hence it follows also that the early blastocyst is not divided into a lower thick-walled 'entodermal' half and an upper 'ectodermal' half, as he suggested.

Selenka's later blastocysts (his two 'young gastrulae') already contain entodermal (?) cells massed at one pole of the egg. From these masses proliferation of flat cells are seen advancing along the blastocyst wall. Little or no differences exist in the cells of the ectodermal wall, the polarity of the egg being indicated merely by the position of the entodermal cells. The ectodermal cells are represented by the author rather ideally as a reconstruction, so that I am at a loss to make accurate comparison with my material. In my collection I find several eggs of batch No. 144 (fig. 27) which show a massing of cells at one pole, though not in the numbers represented by Selenka's young gastrulae. On the whole I believe these two specimens of Selenka to be badly shrunken. Hence, also, his attempt to figure out the axis of the gastrula from the shape of one of his specimens must be characterized as futile, for the differences in the several diameters of the blastocyst were one of chance merely. The blastocysts secured by me are mainly spherical;

where they deviate from this shape in the preparations they have suffered collapse to that extent. In the older of Selenka's two gastrulae the enormous 'Perivitellinraum' between the blastocyst and its albumen layer is to be regarded with suspicion. I have never seen a blastocyst, the cells of which were not in close contact with the albumen layer. Judging from the quantity of albumen remaining in Selenka's figure 1, Tafel 18 (although it is a rough criterion at best) I regard his older gastrula there figured to be a shrunken specimen of a stage just preceding my own No. 40 (4), shown in outline in text figure 6 *D* and in detail in figure 32; and this in turn is a stage just preceding Selenka's almost complete bilaminar blastocyst which he shows in figure 3, Tafel 18.

It is evident then, that Selenka did not, on account of a lack of the requisite stages, hit upon the true method of entoderm formation in the opossum.

b. *Entoderm formation in Dasyurus*

In Hill's account of the history of the blastocyst of Dasyurus he gives us a new method of entoderm formation in mammals, and his interpretation of the facts as he observed them in Dasyurus finds confirmation in similar conditions independently discovered by Patterson ('10) in the embryo of the Tatusia.

In Dasyurus there appears in the still unilaminar wall of the blastocyst, when this has attained a diameter of nearly 4.0 mm., a number of modified ectodermal cells, the 'entoderm mother cells.' These produce the entodermal cells by a process of proliferation and migration on to the inner surface of the blastocyst wall. They appear at the formative pole of the blastocyst and at an early stage are united into a fenestrated tissue by means of pseudopodial prolongations. Eventually they form the entire inner lining of the blastocyst.

Hill's conclusions are borne out by young blastocysts of other Australian forms. In all cases the germ layer formation differs essentially from that obtaining in Eutheria in the absence of the morula stage. That the same facts are substantially true in the development of the opossum I now propose to show.
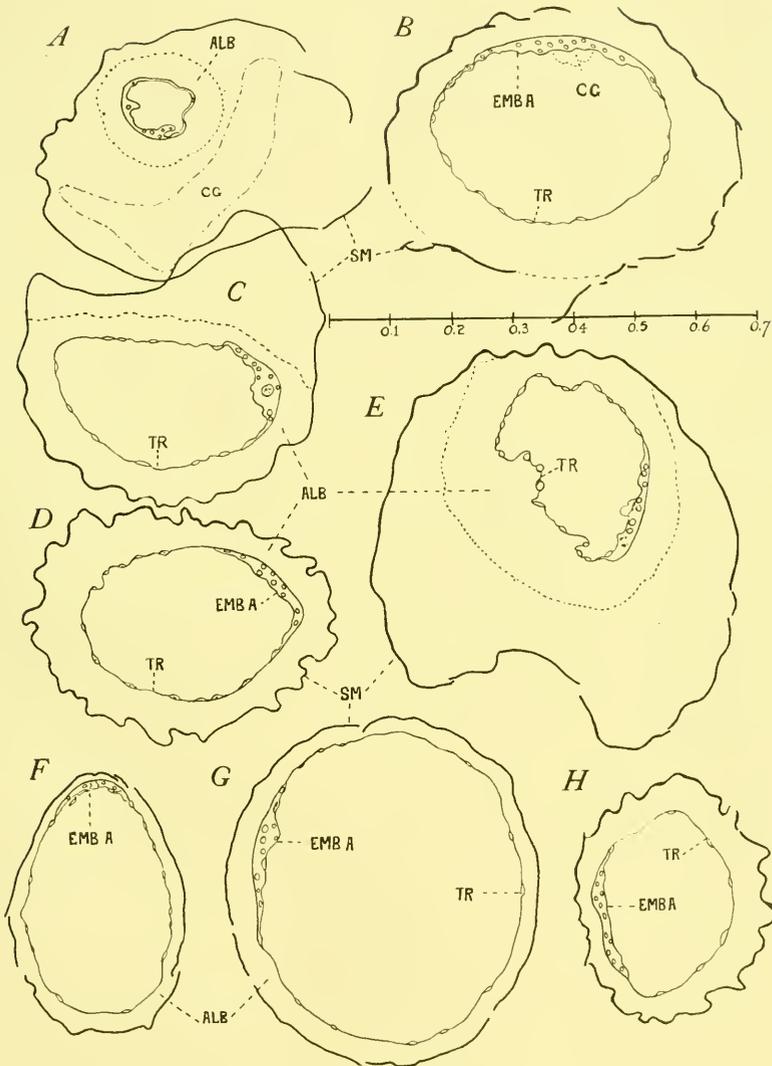
Fig. 6 Sections through the embryonic area of blastocysts at the stage of entoderm formation ($\times$ 90). *A*, No. 144 (1) d; *B*, No. 40 (2); *C*, No. 40 (3); *D*, No. 40 (4); *E*, No. 40 (1); *F*, No. 43 (9); *G*, No. 43 (7); *H*, No. 43 (8). *ALB*, albumen; *CG*, coagulum; *EMB.A*, embryonic area; *TR*, extra-embryonic area; *SM*, shell membrane. The albumen is seen to be absorbed more rapidly over the embryonic area. In *A* and *E*, the shell membrane is misshapen and a large space is left between it and the albumen layer.

c. *Formation of entoderm in the opossum*

The conclusions which follow are based upon four blastocysts of No. 40 and three of No. 43 and corroborative evidence is furnished by a study of the somewhat transitional stages of eggs in batch No. 144. From a study of these blastocysts the conclusion is inevitable that entoderm formation in the opossum proceeds essentially as in Dasyurus, Macropus and Parameles as described by Hill ('10), namely by a proliferation from specialized cells of the unilaminar blastocyst.

An inspection of text figure 6 and figures 27 to 38 will show that at this stage the formative or 'embryonic' area of the blastocyst is sharply marked off from the extra embryonic region. The cells of the former vary from cubical to somewhat elongated, those of the latter are greatly attenuated. In cross-section through the center of the embryonic disc this is seen to occupy from one-fifth to one-third of the circumference of the section, but on account of the great thinness of the non-formative cells, the volume of this region is greater than that of the non-formative.

At different points in the embryonic portion of the blastocyst wall certain cells appear which stain more darkly than cells surrounding them. These show a tendency to push out from their place in the wall in quite the same manner as described by Hill for similar cells in sections of blastocysts of Dasyurus. Inasmuch, furthermore, as these cells are seen frequently to divide and give off dark-staining entodermal cells on to the inner surface of the embryonic disc, we may conclude that we have here the counterparts of Hill's 'entoderm mother cells.'

This differentiation of entoderm mother cells into primitive entoderm does not proceed as fast as the multiplication of all of the cells in the embryonic disc. For scarcely a section can be taken through this region without cutting through one or more cells in mitosis. As a result, the area becomes at first densely studded with nuclei (fig. 30) and later the cells may be crowded in a flattened mass three cells deep (fig. 36). Sections of such blastocysts are almost exact copies of the corresponding stage figured by Hill ('10) of blastocysts of Macropus. The

innermost cells in the last mentioned stage are clearly primitive entoderm. From the margins of the discs entodermal cells may be seen migrating out beneath the wall of the blastocyst. There can be no doubt from this brief statement, that entoderm formation proceeds in the opossum very similarly to the method obtaining in the Australian marsupials. Further details will now be mentioned.

The youngest blastocysts to give any indication of cell proliferation as just described are those of batch No. 144 (fig. 27). The blastocysts contain somewhat over 100 cells. Very little albumen has been absorbed and the cells of the non-formative region have not become much flattened; hence, these specimens are only a little advanced over the 50- to 60-celled blastocysts described in the preceding section. In figure 27, which has formative cells on the reader's left, a large cell is dividing and other cells proliferating from the wall at other points. These latter appear in the figure as mere included cells, but a study of the series of sections shows them to be attached to the wall. The specimens, though poorly fixed, are introduced here because they are clearly somewhat advanced in the direction of the older blastocysts now to be described. These latter are well fixed and for the most part excellently stained preparations.

Specimen No. 43 (9) shows the fewest entodermal cells. Though somewhat crushed at right angles to the edge of the microtome blade (text fig. 6 F) it is otherwise well preserved and the cellular elements are perfectly stained. A portion of the blastocyst wall reproduced in figure 28 shows an 'entodermal mother cell' (ENT^1) in the wall and also a primitive entodermal cell (ENT), both stained darker than the other cells of the blastocyst. The former cell shows a tendency to pull from its place in the wall in precisely the manner illustrated by Hill in his figure 75, Plate 8. In surface view this cell (ENT^1 fig. 28) would appear underneath the adjoining cell of the blastocyst wall.

More cell elements are seen in specimen 43 (8), figures 29 and 30. The primitive entoderm cells (ENT in the figures) are mostly in close contact with the ectodermal cells of the wall.

At ENT^1 an entodermal mother cell has apparently just divided. If we conceive cell ENT^1 of figure 28 as dividing across its long axis (that is obliquely to the surface of the blastocyst), we should have a condition exactly like cell ENT^1, figure 29. At M figures 29 and 30, a large cell is in mitosis. Its staining qualities, however, do not as yet indicate its entodermal nature.

Another large cell is in mitosis in a section of No. 40 (2), figure 31. The spindle of this cell (ENT^2) is oblique to the surface of the blastocyst. At other points (O) there are elongated cells and nuclei with their long axes oblique to the surface. The cell figured at ENT^1 is a just divided primitive entoderm cell; and others are shown at ENT.

A section through the embryonic area of No. 40 (4), illustrated in figure 32, shows at ENT^1 a large darkly-staining cell, which one might interpret as an entodermal mother cell that has left its position in the wall of the blastocyst and has migrated to the inner surface. The section also shows several primitive entodermal cells, clearly darker staining than the overlying cells. Blastocyst No. 40 (1) contains several interesting cases of primitive entoderm cells (figs. 33 and 34) which give every indication of a tendency to migrate from the original position within the wall, so as to underlie the superficial cells. The cell marked ENT^1, figure 34, is an especially striking case in point. Yolk granules (Y, figs. 29, 32 and 33) are of frequent occurrence in these blastocysts, chiefly in the formative region, where, indeed, the only cells thick enough to hold a yolk granule are situated.

A blastocyst containing a large retarded blastomere is shown in figures 37 and 38. The cell is situated in the blastocyst wall within the formative region, which is rather limited in superficial extent. The large cell is almost covered with other cells proliferating from it. The presence of large nuclei in the formative region of other blastomeres indicates that retardation in division of cells is not uncommon.

The cells of several of the blastocysts just mentioned, show a marked tendency toward rapid multiplication in the formative region, so that this area becomes densely studded with nuclei

(figs. 29 and 32). All of the cells multiply faster than the ento-
dermal cells differentiate and as a result the cells become massed
and the embryonic area tends to thicken. The process is con-
tinued until the embryonic disc is about three cells deep (figs.
35 and 36) The innermost of these cells are clearly entodermal.
From the margin of the disc, entodermal cells may be seen to
migrate out in an effort to complete the lining of the blastocyst.

If one compare sections through the embryonic area of the
opossum blastocysts which have just been described with sec-
tions through corresponding stages of Macropus and Parameles,
as figured by Hill, the similarity is very striking. In all cases
the area tends to be much thicker than that of Dasyurus. This
agreement of the opossum with Macropus and Parameles is corre-
lated with the agreement in size of blastocysts as compared with
the blastocysts of Dasyurus at the time of entoderm formation;
for this process in Dasyurus is preceded by a period of extensive
growth. The bearing of this on the subject under discussion
will appear presently.

Surface views of this stage of the opossum blastocyst were
not studied, since the technical difficulties of making surface
preparations are almost insurmountable on account of the small
size of the opossum blastocyst and the interference of the super-
imposed albumen. The corresponding stage in Dasyurus has
a diameter of 4.0 mm. (eight to ten times that of the opossum
blastocyst) and the albumen has long since disappeared (K and
J, text fig. 4). Inasmuch as surface views were not available,
the entoderm mother cells were not studied in toto, hence it
was not possible to determine whether or not these cells possess
pseudopodial prolongations and are capable of amoeboid move-
ments. Several cells seen in section, however, as pointed out in
detail above, strikingly resemble those shown by Hill in draw-
ings of sections through the embryonic area and interpreted by
him as primitive entoderm cells. These facts as they occur in
the opossum are presented as observed, and the interpretations
of the conclusions suggested are those which a careful study
of the material seems fully to justify.

d. *Comparison of early and later blastocysts and growth of the egg*

A comparison of figures *H* and *J* (text fig. 4) will show the reader at a glance the changes which the blastocyst, the albumen and the shell have undergone from the 50-celled stage to the time that the entoderm formation is initiated.

The entire egg has grown comparatively little in size—from a diameter of 0.40 mm. on the average to about 0.50 by 0.60 mm. in the largest specimen (text fig. 4 *J*). This increase is due largely to the turgidity of the entire structure, the liquid within the ovum pressing against the plastic wall, which gradually responds. In the fresh state and in alcohol the blastocyst eggs in both stages are practically spherical. The blastocysts shown in figure 6 differ more in the preparations than they did in the fresh state, on account of differences in amount of shrinkage. Measurements of the thickness of shell membranes resulted as follows: No. 40, 0.0035 mm.; No. 43, 0.0027 mm.; No. 144, 0.0023 mm. Practically no change in thickness of shell has taken place since the 16-celled stage.

The blastocysts themselves vary more in diameter than do the shell membranes, chiefly because of the differences in quantity of albumen still left unabsorbed. It will be noticed that absorption of albumen proceeds most rapidly in the region of the blastodisc, less at the opposite pole. This is as one would expect from the very apparent difference in protoplasmic mass as well as from differences in metabolic activity of the cells at the two poles. The absorption of albumen continues in this proportion; so that in bilaminar blastocysts, when the formative region comes to lie against the shell, the non-formative region may still have a greater or less thickness of albumen separating it from the shell (text fig. 4 *L*). The ratio of activity of the formative cells (and the consequent thickness of the embryonic area) to quantity of albumen absorbed holds true only in general terms. Thus *B*, text figure 6, represents an egg with thickened embryonic disc whereas considerable albumen is left in the egg; figure *F*, on the contrary, shows little albumen as well as few cell elements in the formative area.

Included yolk and coagulum are still seen in several of the specimens, and large yolk granules, such as observed regularly in the earlier stages, still occur within the blastomeres (figs. 29, 32 and 33). Inasmuch as the thickened area of these blasto- cysts is very clearly the formative region, the question arises: which portion, the thicker or the thinner half, of the earlier (50-celled) blastocysts is formative and which the non-formative portion? Polar differentiation in the opossum. it will be recalled, first manifests itself by a more rapid thinning at one pole of the blastocyst of 50 or more cells. It will be recalled also, that Hill, interpreting Selenka's two earliest blastocysts in the light of the cleavage process in Dasyurus, considers the larger, more yolk- laden, cells of the lower pole as non-formative; the other as forma- tive. I venture the assertion that the thicker-walled pole is the formative region (compare text figs. 5 and 6). The thinning of half or more of the blastocyst is simply the accompaniment of the differentiation of the embryonic disc. Blastocysts of No. 144, although shrunken, are especially instructive in this con- nection, since several of them are clearly a little more advanced than those described in the last section. From figure 27 and text figure 6 A, the change here indicated may well be seen. One region of the blastocyst (to the right in the figures) is more attenuated and larger in extent than the other. The differen- tiation has gone further than in any blastocyst shown in figure 5, and figure 27 is only a little more advanced. The whole series is so nearly complete as to justify the conclusion that, once begun, the differentiation of the formative region is pro- gressive.

From the specimens described in this section, therefore, there can be no doubt whatever that, in common with other mar- supials, the opossum lacks the morula stage in its development. It is furthermore clear that cell proliferations do occur from the blastocyst wall in the region of the blastodisc and that these proliferations result in the formation of the definitive entoderm.

GENERAL SUMMARY

1) Maturation of the opossum egg begins in the ovary, where, as in other mammals, the first polar body is given off (fig. 2, pages 15–16).

2) An average of twenty-two eggs are shed at one ovulation, but the number may rise to forty-five. This overproduction of eggs is accompanied by a high percentage of mortality at each stage in the development of the opossum, for unfertilized or otherwise abnormal eggs are found in each lot and dead foetuses are of frequent occurrence. On removal of the ovary and uterus from one side at the time of the first ovulation, the remaining ovary may compensatorily double the normal production of eggs at the second ovulation (pages 10–15).

3) Measurements of a dozen full and unshrunken ovarian ova gave an average of 0.165 x 0.135 mm. Unsegmented tubal and uterine eggs in the sectioned and stained preparations measure less, namely from 0.146 x 0.123 mm. to 0.095 x 0.080 mm., or an average from six lots of 0.111 x 0.107 mm. Hence the eggs of the opossum are on the average larger than most mammalian eggs. They are much smaller than the eggs of Dasyurus, which measure 0.27 x 0.24 mm. (fig. 4 and its legend).

4) The ripe egg of the opossum exhibits no evidence of polarity except that afforded by the position of the polar body and chromosomes of the egg nucleus. No polar concentration of deutoplasmic substance is ever encountered in the unsegmented opossum egg. The yolk granules or yolk spherules are for the most part situated in a peripheral or submarginal region, just beneath a thin yolk-free layer of granular cytoplasm (figs. 1, 3, 6, 8; pages 15–18).

5) Insemination occurs in the oviduct, where doubtless the second polar body is given off; neither process was, however, actually observed. After insemination the egg receives, through the secretive activity of the gland cells lining the oviduct, the secondary egg envelopes:—albumen and shell membrane. The albumen is homologous with the albumen covering of other Amniota; the shell membrane is homologous with the shell of Monotremata (figs. 5 and 6, pages 18–20).

6) When the eggs pass from the oviduct the shell membrane is thin and delicate, measuring 0.0012 mm. in thickness. It grows thicker rapidly, soon attaining a maximum which remains practically constant until the didermic blastocyst is fully formed. There is no progressive growth in thickness of shell membrane as in Dasyurus. That different batches of eggs, even in the same stage of advancement, vary considerably in this respect may be seen from the following table (figs. 4 and 5, page 19).

TABLE 2

NORMAL STAGE OF EACH LOT OF EGGS	NO. OF LOT	AVERAGE THICKNESS OF SHELL
Unsegmented..........................	54 & 58	0.0012
	52	0.0034
	46	0.0055
4-celled.............................	81	0.0022
8–18-celled..........................	85	0.0033
	117	0.0030
Just completed blastocyst.............	50	0.0034
	88	0.0045
Early bilaminar blastocyst............	144	0.0023
	40	0.0035
Completed bilaminar blastocyst........	43	0.0027
	55	0.0033
	82	0.0048

7) Uterine eggs in early cleavage measure from 0.4 to 0.5 mm. through the shell membrane, or an average of about 0.45 mm. Hence the albumen layer is on the average 0.15 mm. in thickness. This thickness is maintained until the fully formed blastocyst begins to grow at the expense of the albumen. The egg grows very little in diameter of shell, being only 0.6 mm. in diameter at the time entoderm formation sets in or 0.7 to 0.9 mm. at the completion of the didermic blastocyst, when nearly all the albumen has been digested (fig. 4, pages 20 and 56).

8) The pronuclei are fully formed when the egg enters the uterus. At first they occupy a position within a yolk-free area at one pole, but soon migrate to the center, where the first cleavage spindle takes its position (figs. 4, 6 to 8, pages 21–22).

9) The first cleavage plane divides the egg into approximately equal halves. At this time the elimination of yolk takes place. This occurs, not by the extrusion of a deutoplasmic mass at one pole, but by the elimination from the entire periphery. The two blastomeres form a new membrane just within the yolk-laden submarginal layer, leaving masses of cytoplasm rich in yolk between the blastomeres and the zona pellucida. In this condition the eliminated material is later found in the cleavage cavity of every stage until absorbed. The two blastomeres exhibit no evidence of polarity. The yolk remaining within the blastomeres in the 2-celled, as well as in later stages, occupies a submarginal zone as in the unsegmented egg (figs. 7–10, pages 22–24).

10) The second cleavage plane is again meridional and at right angles to the first. The four resulting blastomeres are spherical and shift their position until the pairs come to lie at right angles to each other. From this time on the cleavage is indeterminate, as in the Eutheria (text fig. 1 and figs. 11–18, pages 24–28).

11) As cleavage proceeds the cells migrate to the periphery and become closely appressed against the zona pellucida or, if this has disintegrated, against the albumen layer. As a result, at the end of the fourth cleavage (and sometimes before this) the cells are arranged in the form of a sphere enclosing a considerable cleavage cavity, in which the eliminated yolk and co-agulum are to be found. The cells of the 8- and 16-celled stages are practically alike in every particular (text figs. 2 and 3, figs. 19–24).

12) A study of an unbroken series from the 2-celled to the 18-celled stages, together with a fairly complete series up to the time of entoderm formation, makes it highly probable that the cells of each hemisphere are lineal descendants of one or the other of the two blastomeres of the 2-celled stage. The cells of the two hemispheres are destined to form the embryonic and the non-embryonic regions, respectively, of the blastocyst. This interpretation is supported by a full series of wax models of the cleavage stages. Hence, also, the unsegmented opossum ovum possesses a potential though concealed polarity and con-

sists of a formative and a non-formative portion. This inter-
pretation homologises the opossum egg with that of Dasyurus,
in which polar differentiation is structurally evident throughout
early cleavage (text figs. 1, 3, 4 and 6, pages 39–40).

13) Since the blastocyst of the opossum is already anticipated
in the 16-celled stage by the arrangement of the cells into a
hollow sphere, the blastocyst is completed at an early stage,
that is, in eggs of about 40 cells. By this time the ovum has
grown very little, the albumen layer being as thick as in previous
stages. The corresponding stage of Dasyurus contains about
125 thin cells which lie immediately against the shell membrane.
About this time polar differentiation is manifested for the first
time in the history of the opossum egg as a thinning of the cells
at the pole destined to be the extra-embryonic area of the defini-
tive vesicle. Included cells are almost always present in the
early blastocysts (figs. 4, 5, 6, 26, 27, pages 42 and 49).

14) The morula stage is absent in the opossum egg. The ento-
derm is formed by the proliferation of specialized 'entoderm
mother cells' which appear only in the embryonic area. The
entoderm mother cells may or may not migrate from their posi-
tion in the wall of the blastocyst, but in either case they give off
by division primitive entodermal cells which spread out at first
immediately beneath the embryonic ectoderm and later beyond
this area, until they completely line the entire vesicle. Ento-
derm formation sets in when about half the albumen is absorbed
and the blastocyst is 0.6 mm. or less in diameter. Hence there
is no such great period of growth as obtains in Dasyurus. The
didermic blastocyst of the opossum is completed when the egg
is only about double the original size as measured through the
shell membrane (text fig. 4, figs. 28–38, pages 49–57).

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PLATE I

EXPLANATION OF FIGURES

1 Tubal ovum, No. 76 (3). The distribution of yolk spherules is not quite typical, an unusual number of them having invaded the central, generally yolk-free zone ($\times$ 600).

2 Ovarian egg, No. 21 (20), showing discus proligerus (*DP*), polar body (*PB*), and chromosomes of the egg nucleus (*CH*). The zona pellucida is best seen at the lower margin of the egg. The section is taken somewhat to one side of the center ($\times$ 430).

GENERAL ABBREVIATIONS

CG, coagulum
CH, chromosomes of egg nucleus
EMB.A., embryonic area
PB, polar body
SM, shell membrane

TR.ECT, trophoblastic ectoderm
Y, yolk
ZP, zona pellucida
ZR, zona radiata

Note: The figures in these plates were drawn by Miss Aimèe Vanneman, technician in the School of Zoology, the University of Texas. The outlines for the drawings were made with the aid of a Bausch and Lomb drawing apparatus.
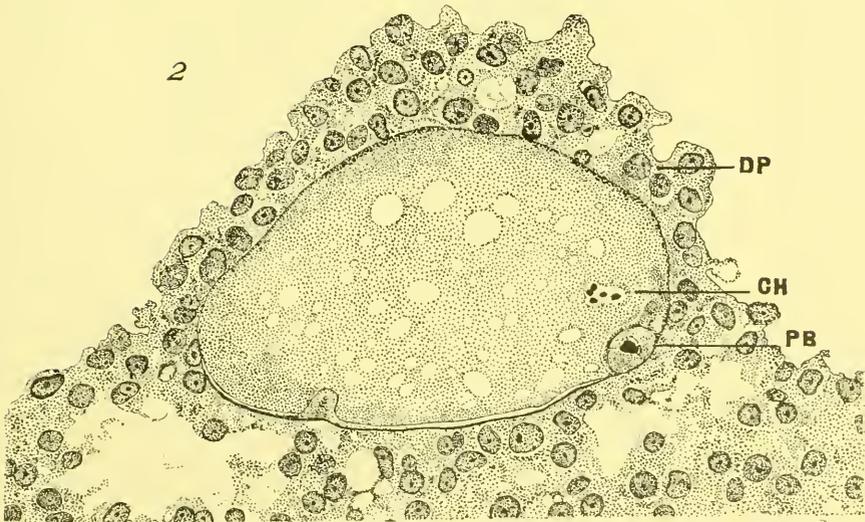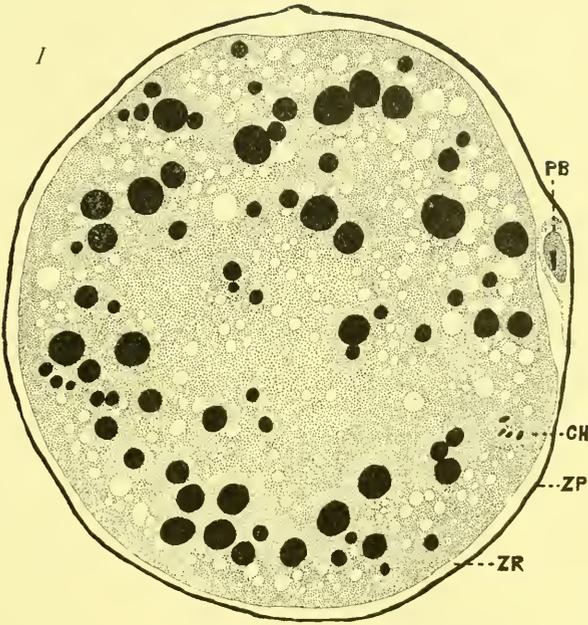
3   Tubal ovum, No. 56 (10).   The yolk-free peripheral and central zones and the yolk-laden submarginal zone are well shown ($\times$ 607).

4   Uterine ovum, with pronuclei, No. 54 (2).   The specimen is somewhat abnormal in the possession of the third body (A) adjacent to one of the pronuclei ($\times$ 790).

65
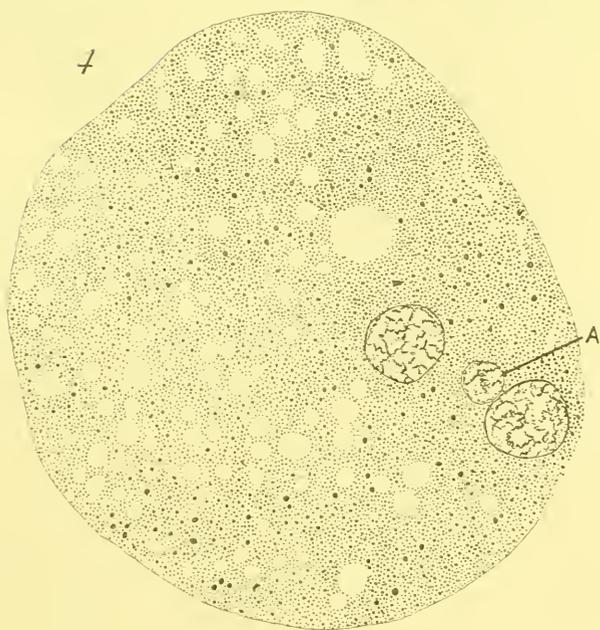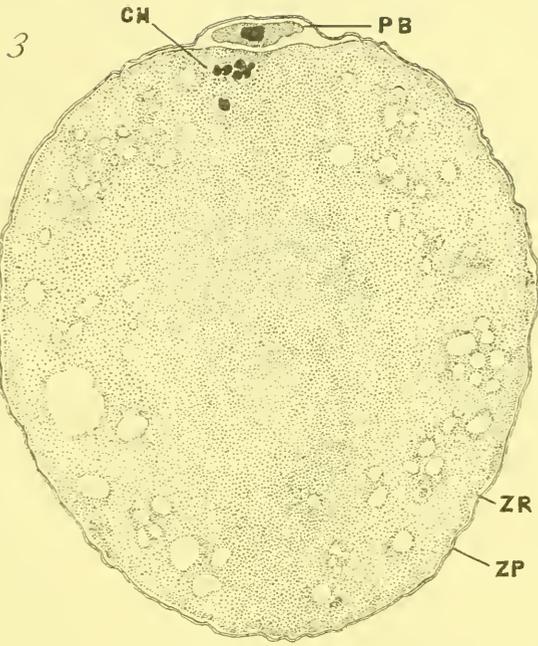
5   Unfertilized uterine egg, No. 58 (2), just after it entered the uterus.   On account of its thinness, the shell membrane has shrunken considerably.   The large yolk spherule is seen at one pole.   The egg is slightly shrunken away from the zona at one side ($\times$ 525).

5

SM

ALB

PB

ZP
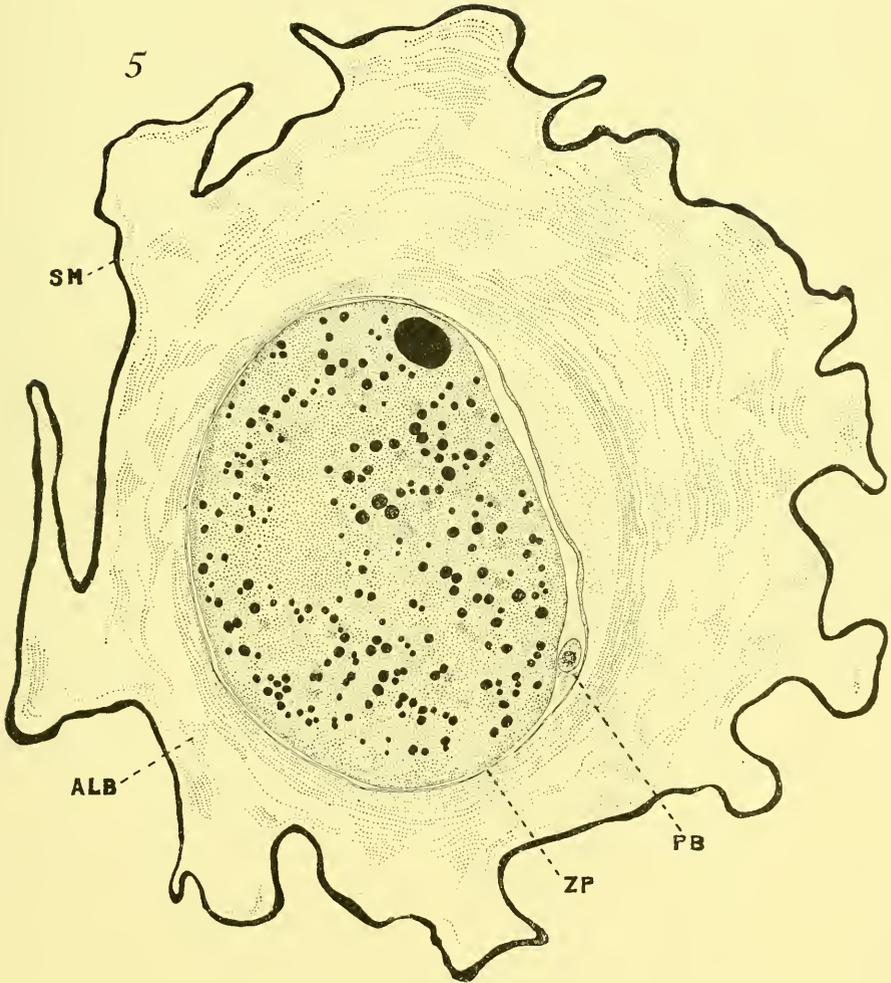
6   Uterine ovum, No. 52 (7), with pronuclei near the center.   The pronuclei are not in the same section in the series, but are here represented together, the drawing having been made by superimposing the two sections containing the pronuclei ($\times$ 790).

7   Fertilized uterine ovum, No. 54 (4), with chromosomes of first cleavage spindle.   An unusual preponderance of yolk vacuoles is to be noticed in the lower hemisphere of the egg ($\times$ 790).

6

7
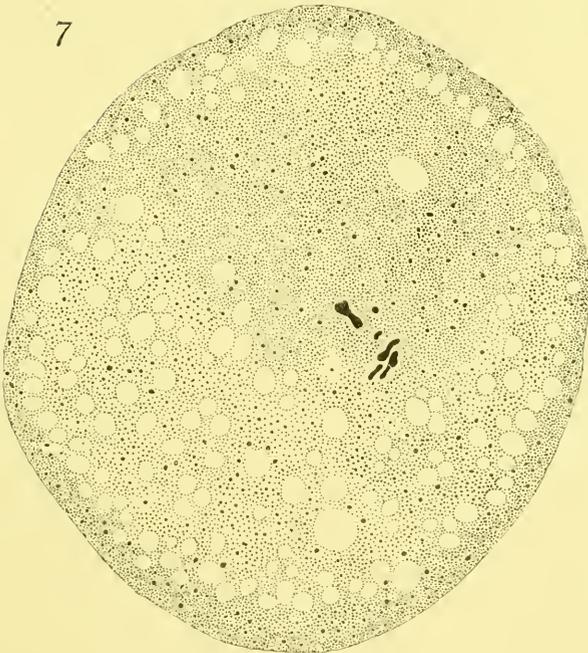
PLATE 5

8   Portion of first cleavage spindle in uterine egg No. 52 (3) ($\times$ 790).

9   2-celled stage showing elimination of yolk; egg No. 50 (6).   The cell to the left has separated from the eliminated material and rounded off.   The cell to the right has not yet separated, but the line of separation is indicated by the dark granular zone (CM) ($\times$ 405).

10   2-celled egg, No. 46 (3).   The yolk has been eliminated; the cell to the left has not yet rounded off.   The yolk granules are again assuming a submarginal arrangement in the blastomeres ($\times$ 405).
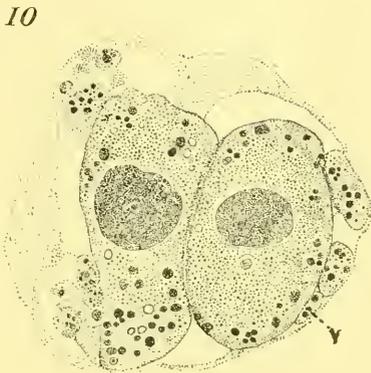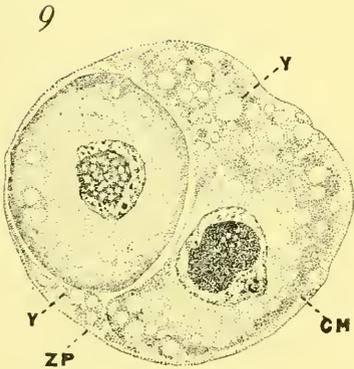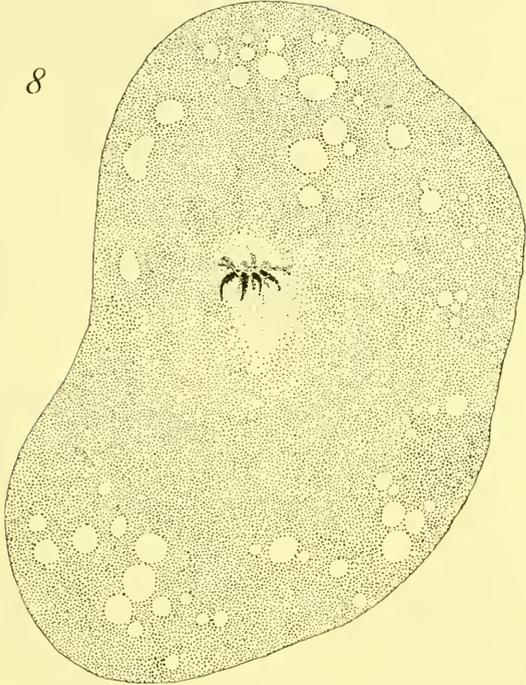
11 and 12   Two sections of 4-celled egg No. 81 (5), showing relative position of the two pairs of blastomeres.   The configuration of the shell membrane (SM) and the mass of albumen (ALB) will enable the reader to determine the axes of the pairs of blastomeres.   It will be noted that these are nearly parallel ($\times$ 125).

13 and 14   Two sections of 4-celled egg No. 81 (11).   The blastomeres have shifted more than those of figures 11 and 12 (text figure 1 B).   The cells are relatively small ($\times$ 125).

15 and 16   Two sections of 4-celled egg No. 81 (5). showing the two pairs of blastomeres almost at right angles to each other.   Compare text figure 1 A ($\times$ 125).

CARL GOTTFRIED HARTMAN
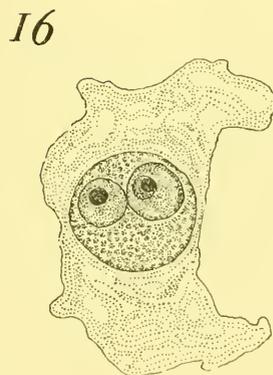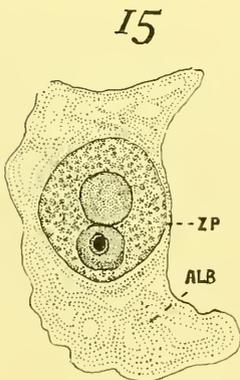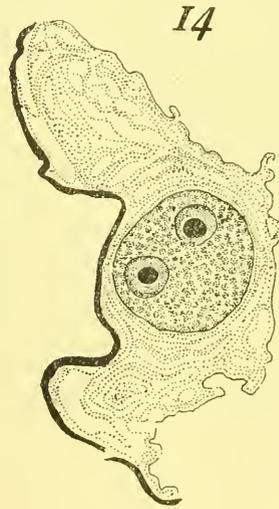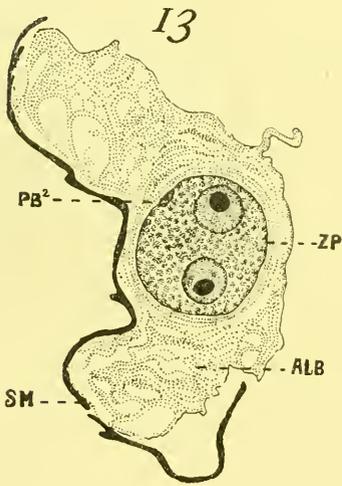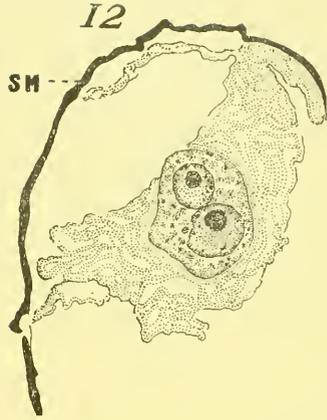
17 Section of a 4-celled egg No. 81 (2), showing characteristic appearance of yolk in this batch of eggs. In some of these eggs the blastomeres are without clear-cut cell membranes ($\times$ 390).

18 Section through three blastomeres of the 4-celled egg No. 83 (7). The blastomeres of the 4-celled stages are in all cases so oriented that no section contains more than three cells ($\times$ 390).

19 Section through a 7-celled egg, No. 85 (7). The tendency of the cells to flatten out against the zona is already manifested at this stage. The peripheral arrangement of yolk remaining within the blastomeres is well shown ($\times$ 390).

20 Section through 9-celled egg, No. 85 (3). The blastomeres are in contact with the albumen layer. The cells are large and little yolk is seen in the cleavage cavity. The vacuolization in the inner margins of cells is due to breaking down of deutoplasmic content of cells in this region. The yolk has not all as yet been crowded into the cleavage cavity ($\times$ 390).

21 Section through the four blastomeres of the 12-celled egg No. 85 (18) b. The blastomeres are numbered to correspond to the figures employed in text figure 3 B. Cells 5 and 6 are in mitosis. Unlike the two preceding specimens, the cells of this egg are not yet in intimate contact with the zona ($\times$ 390).

22 Section through the 16-celled egg No. 85 (2). The centrifugal migration of the cells and their flattening out against the zona are well shown. The yolk and coagulum occupy the cleavage cavity ($\times$ 390).

74
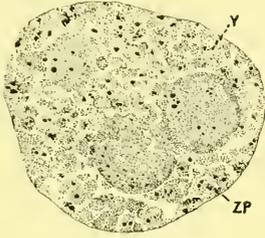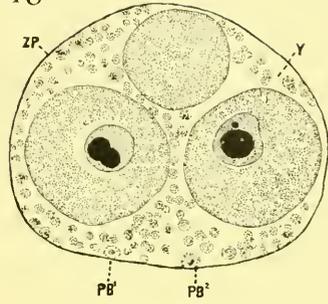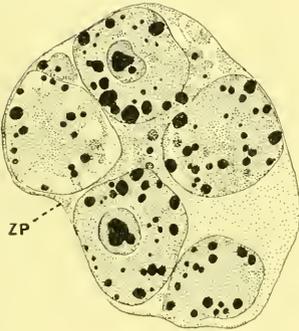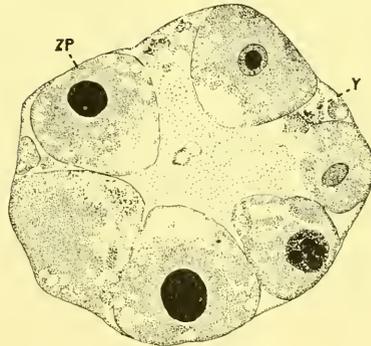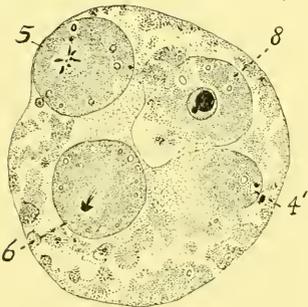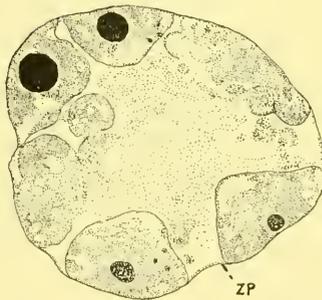
23 and 24   Sections through the 16-celled eggs; Nos. S5 (11) b and S5 (11) a, respectively, to show the relative distribution of yolk granules within the blastomeres and in the cleavage cavity.   As in figures 19–22 the blastocyst formation is anticipated.   The blastocyst will be completed when the gaps (i, i) have been filled by the continued flattening and multiplication of the cells ($\times$ 390).

25   Section of 52-celled completed blastocyst, No. 50 (3) a, showing included deutoplasm and coagulum in cavity.   The large cell in upper portion of figure is one of four retarded blastomeres situated at this pole ($\times$ 563).
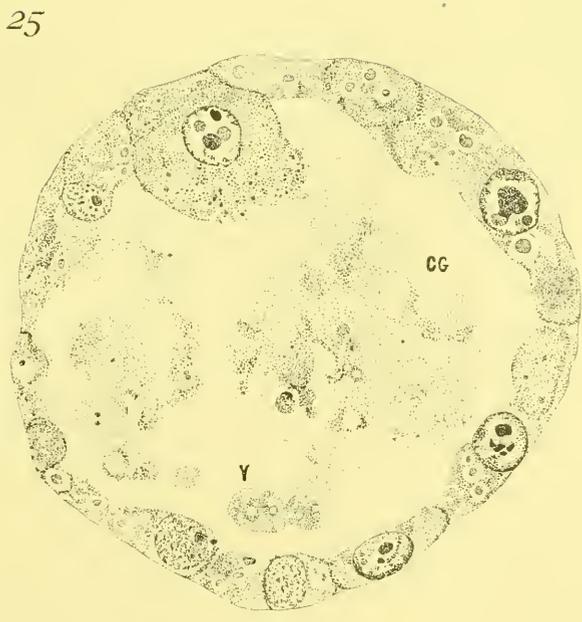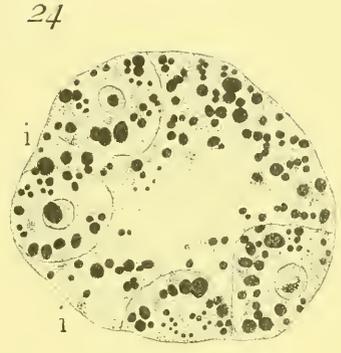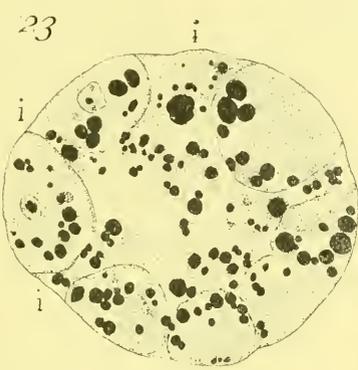
26 Section of blastocyst No. 50 (3) b, to show distribution of yolk granules. The yolk granules in the cleavage cavity are to be found chiefly within the cytoplasmic fragments and the included nucleated cell (C). Much yolk is still within the blastomeres (× 540).

27 Section of blastocyst No. 144 (1) d, somewhat more advanced than those shown in figures 25 and 26 (A, text figure 6). The specimen is somewhat shrunken. The non-formative area (to the reader's right) has become considerably attenuated. A study of the series from which this section is taken shows that the apparently included cells are attached to cells situated in the wall of the blastocyst (× 375).
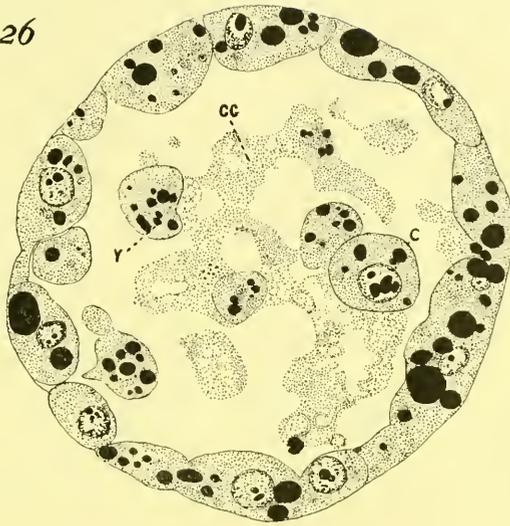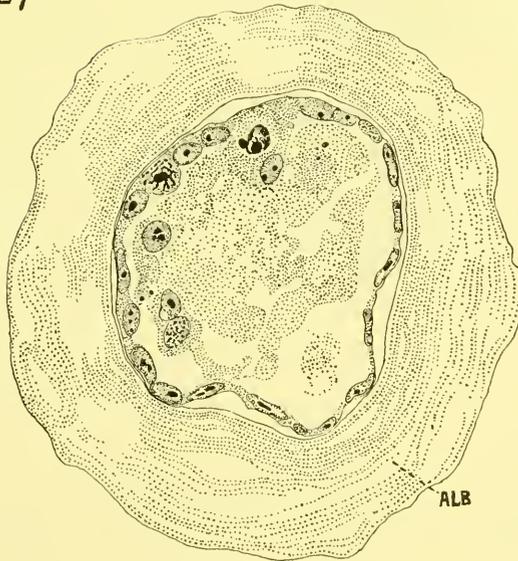
28   Portion of formative region of blastocyst No. 43 (9).   *ENT*,[1] entodermal mother cell in the blastocyst wall; *ENT*, primitive entodermal cell.   Both cells are much darker than the adjacent ectodermal cells.   See *F*, text figure 6, for outline sketch of entire section of blastocyst.

29 and 30   Adjoining sections through the embryonic area of blastocyst No. 43 (8), shown in outline in *H*, text figure 6.   *ENT*,[1] primitive entodermal cell dividing from an entodermal mother cell; *M*, large cell in mitosis.   This cell contains a large fat spherule shown in section in figure 30.

31   Section through embryonic area of specimen No. 40 (2).   (Cf. outline sketch *B*, text figure 6.   *ENT*,[1] entoderm cell being given off from entodermal mother cell situated in the wall of blastocyst; *ENT*,[2] large cell dividing obliquely to surface; *O*, elongate cells and nuclei, with axes oblique to surface of blastocyst.

32   Section through embryonic area of *D* text figure 6, specimen No. 40 (4).   *ENT*[1] entodermal mother cell which has migrated from its position in the wall of the blastocyst.

33 and 34   Two sections of blastocyst No. 40 (1), shown in outline in *E*, text figure 6.   *ENT*[1] primitive entoderm cell just divided from entodermal mother cell and pushed out underneath the superficial ectodermal cell.

35   Section through middle of the embryonic area of blastocyst No. 43 (7), shown also in *G*, text figure 6.   The area has thickened on account of rapid cell division at this point, nearly every section of the series containing one or more cells in mitosis.
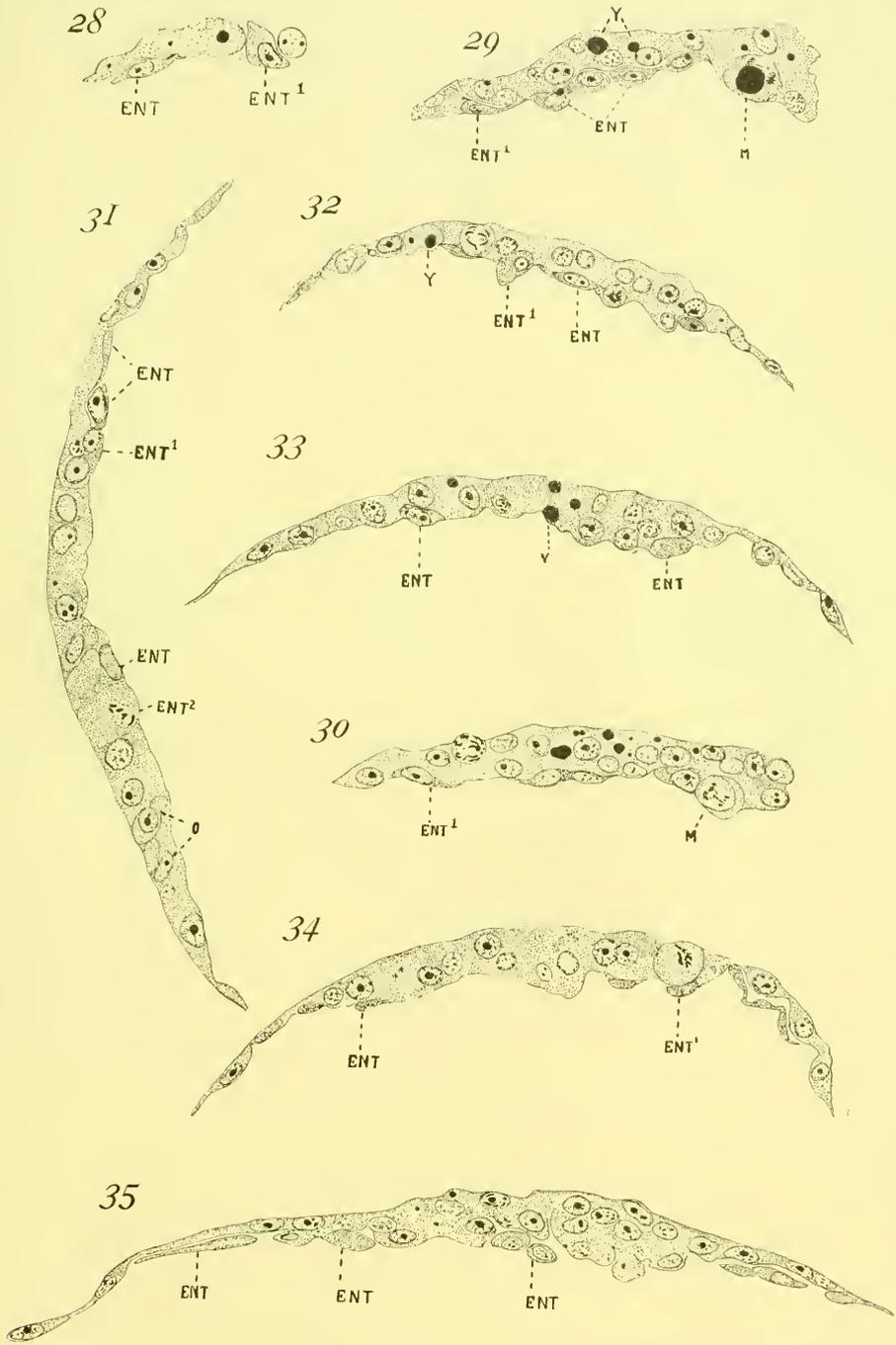
36   The tenth of the series of 40 sections through the same egg of which figure 35 represents the 21st section.   The thickening of the embryonic area is better shown here.   Three cells are in mitosis.   At ENT^1 the entodermal cells are seen to migrate from the margin of the embryonic area.

37 and 38   Sections of blastocyst No. 40 (3)   shown also in C, text figure 6. The embryonic region $(EMB.A)$ is thick, but limited in superficial area. C, large cell, a retarded blastomere; this cell comes to the surface in a number of sections of the series. $TR.ECT$, trophoblastic ectoderm.
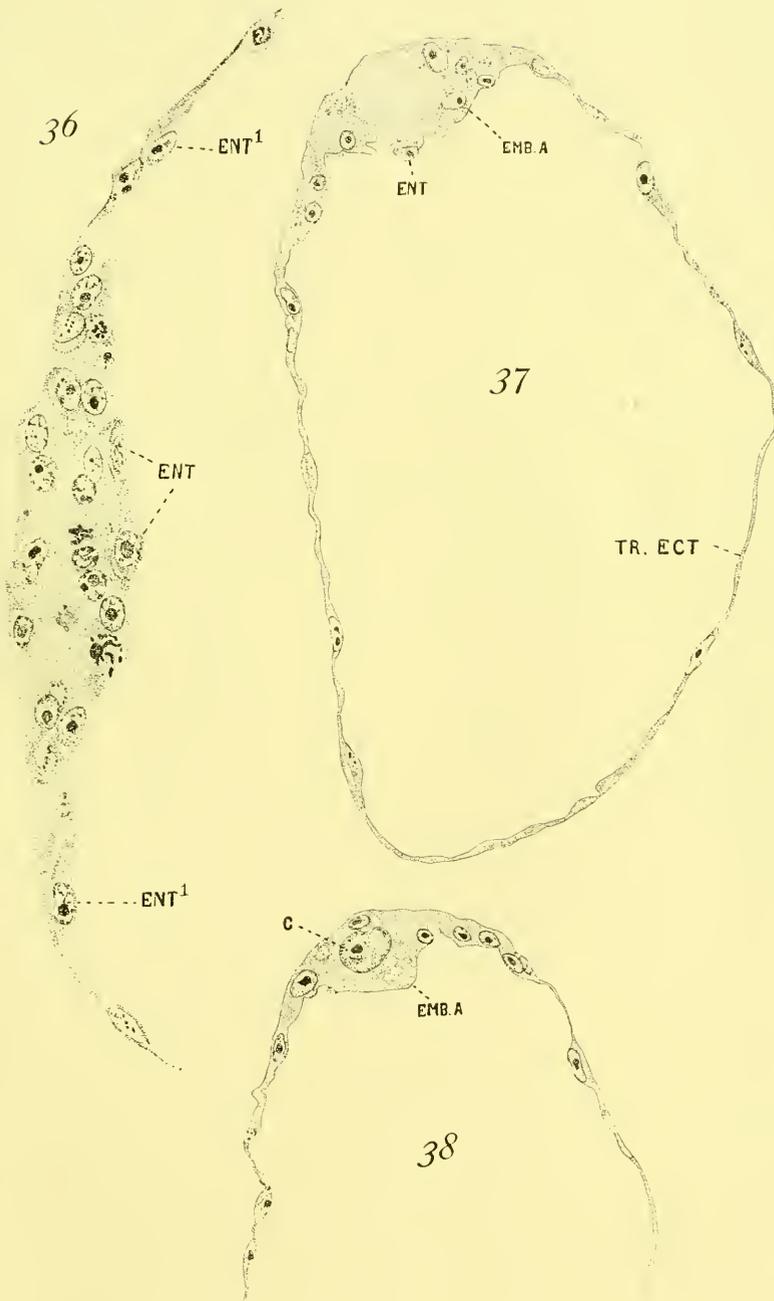
GERM CELLS OF COELENTERATES. II. CLAVA LEPTOSTYLA

GEORGE T. HARGITT

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ELEVEN FIGURES (TWO PLATES)

In part I of this series the oogenesis of one of the campanularian hydroids (Campanularia flexuosa) was described in detail. It was there pointed out that an egg cell arises in the entoderm from one of the ordinary epithelial cells or from a part of such cell as a result of division, and this, it was believed, demonstrated the absence of any differentiation of a distinct germ plasm. A single primordial cell develops directly into a single egg cell without intervening cell divisions, therefore without generations of oogonia and oocytes.

The chromatin of the nucleus of the growing oocyte was partly in a nucleolus and partly in a delicate and fine meshed reticulum. During growth the nucleolus broke into small fragments which passed out into the cytoplasm to form, or to aid in forming, the yolk. Since the growth of the egg began with this emission of chromatin from the nucleus and ceased when this emission had been completed it was clear that such emission had some causal connection with the metabolic activities of the developing egg.

It was also noted that in Coelenterates the place of origin of the germ cells is not now of fundamental importance, since Goette ('07) and others have shown how variable it may be even in different individuals of the same species. There is, however, still some difference of opinion with regard to the origin of the germ cells of Clava and the present report may aid in harmonizing in some measure these divergent views.

In Clava squamata Weismann ('83) described the egg cells as first distinguishable in the entoderm, close to the supporting layer. He suggests a probable ectodermal origin and a migration through

the supporting layer, though he could not demonstrate this. Harm ('02) makes the same claim and also believes the germ cells are present in the larva (planula) and can there be distinguished from other cells. This latter claim is an important one if it can be substantiated, since it would seem to show an extremely early differentiation of the germ cells C. W. Hargitt ('06) in the American species C. leptostyla, finds the egg cells always distinguishable in the entoderm and never originating in the ectoderm. Goette ('07) finds the germ cells to arise in C. multicornis "only from transformed half-entoderm cells."

There is no doubt that the egg cells are usually found, and very easily distinguished as germ cells, in the entoderm against the supporting layer. Figures 3 to 5 show various stages of growth of the eggs in this position. Concerning the origin Weismann claimed the ectoderm on theoretical grounds, his observations not including any demonstrated cases of eggs in the ectoderm. Harm, on the contrary, gives figures of cells in the ectoderm and others in the supporting layer and entoderm of the planula which he says are egg cells. Unfortunately his figures are too small to enable one to determine the characteristics of these cells. His description states only that such cells have a more compact cytoplasm than the adjacent cells.

In figure 1 is represented a cell found in the ectoderm of the polyp against the supporting layer, and figure 2 shows two similar cells within the supporting layer but not quite separated from the ectoderm. These cells are quite similar to the small oocytes shown in figures 3 and 4 in the entoderm. Such cells found in the entoderm can be directly connected, stage by stage, with large egg cells and there is no question of their character; consequently similar cells found in the ectoderm must also be considered as primordial egg cells. As a result there is represented in the material on which this study is based evidence of the origin of some of the egg cells in the ectoderm and their probable migration (fig. 2) through the supporting layer into the entoderm. However, in a great many sections of numerous individuals obtained from two regions (Woods Hole, Massachusetts and Casco Bay, Maine) this was the only one in which

I found egg cells thus arising, so that such is not the usual method of origin. In the same individual just referred to, most of the egg cells were taking their start in the entoderm, figure 3, for example, being a drawing of a section of the same individual in which numerous eggs were forming in the entoderm. From an examination of many individuals it is clear that the common method of origin of the egg cells in Clava leptostyla is that stated by Goette for C. multicornis, viz., by a division of the entoderm cells in the base of the pedicel of the gonophore, or of the adjacent polyp wall. Clava, therefore, agrees with Campanularia in the method of origin of the egg. But in Clava such cells may occasionally form in the ectoderm and pass through the supporting layer into the entoderm, just as Weismann believed might be the case.

In forms like Clava and Campanularia, where each primordial germ cell develops directly into a single mature egg, I have found that the only sure sign for distinguishing such primordial cells is the appearance of the nucleus. This is always large, and at an early stage the chromatin is arranged in loops disposed in polar fashion toward one side. The cytoplasm is more compact and finely granular and takes a deeper stain with hematoxylin and basic stains than do other cells except gland cells. Only when such differences can be made out is one able to differentiate between the germ cells and the adjoining cells. This distinction is evident as soon as the division of the entoderm cell has taken place and the nucleus has been reformed. The more usual method of distinguishing germ cells from others is the close packed, deeply staining, granular cytoplasm, but this by itself can not be depended upon and has undoubtedly at times led to error by mistaking gland cells or developing nematocysts for germ cells.

Concerning the claim of Harm that in C. squamata germ cells could be distinguished in the larval stage (planula) nothing positive can be said since it has not been my fortune to see this species. The same can be said of Gonothyraea loveni in which a similar claim of the differentiation of germ cells in the larva is made by Wulfert ('02). It is, of course, possible that such is the

case in these two species, but it might be expected that closely related forms would show the same thing.   But no one who has studied these or similar forms, whether European or American species, has described or claimed such an early differentiation, save the two authors mentioned.   In the descriptions of both Harm and Wulfert the only evidence presented for identifying certain cells as primordial germ cells was the appearance of the cytoplasm, and I have shown that by itself such evidence is not conclusive and is apt to lead one into error.   The figures of these two authors are not sufficiently clear to show whether other characteristics are apparent.

In Campanularia flexuosa, Tubularia crocea, Pennaria tiarella, Hybocodon prolifer, Cyanea arctica, Aurelia flavidula and other coelenterates I have examined the developing egg and larva without finding germ cells, though there are some cells which may have some resemblance to germ cells.   In Clava leptostyla I have examined young and old polyps, young and old gonophores, and various stages in the development of the egg, and only when the gonophores are about to form, or have formed, are germ cells present.   When present, such cells are found only in the pedicel of the gonophore or in the wall of the hydranth close to the gonophore stalk.   Furthermore, in no case has it been possible to identify germ cells until they have the characters already enumerated as diagnostic.   This is, of course, clearly understandable in those cases where germ cell are derived by division from the general epithelial surface; in such cases the germ cells are not present before this period.   Also in Campanularia and others, where the eggs develop within closed gonophores, there are few interstitial cells, gland cells, or nematocysts and consequently nothing to render the germ cells indistinct, or to confuse with them.   Under such conditions there is slight chance of missing any germ cells which might be present, and their absence is therefore good warrant for denying any early differentiation and segregation.   In the forms mentioned as having come within my own observation I am confident that germ cells are not recognizable in such early stages of development as claimed by Wulfert and Harm.   I have a feeling of skepticism,

therefore, as to the correctness of their interpretation on this point.

In Clava, as well as in Campanularia, another point of some theoretical interest and importance calls for comment. This is in regard to the method of reduction of chromosomes. Beckwith ('09) has shown in Clava, as I have in Campanularia ('13), that when the first maturation spindle forms, the chromosomes are present in the reduced number. This is the usual condition in oogenesis, of course, but as a rule there is a period of contraction of the chromatin into a mass at one side of the nucleus (synizesis) at which time it is supposed that the chromosomes unite in pairs (synapsis). In Campanularia it was found, and figures 1 to 4 here show for Clava, there is no synizesis stage. The only stage which would suggest synapsis is that in which the chromatin is arranged in loops (figs. 1 and 2) and these loops at the time of the first recognizable appearance of the egg cells are already apparently present in the reduced number. This may mean that synapsis does not occur (that is chromosomes do not conjugate) but the chromatin at the time of formation of the chromosomes condenses into half the usual number of bodies. This would indicate a variation in method only and not a difference in effect. But if confirmed it would present evidence against the view of the presence and independence of distinct paternal and maternal chromosomal constituents in each cell.

Synapsis is assumed to represent a fusion of similar chromosomes and in many cases the chromosomes which unite are alike in size and shape, and later these separate. If one half the usual number of bodies are produced in the nucleus, but without the preliminary formation of definite chromosomes, to harmonize this with the prevailing view of the fusion of homologous paternal and maternal constituents would involve the further assumption of a fusion of similar chromatin granules. These would have to be considered as genetically related to granules which entered the cleavage nucleus from the egg and sperm chromatin. While such an interpretation has been theoretically made to explain certain cases of inheritance, as far as I am aware no one has observed any differences in chromatin granules which would

warrant their identification. On the basis of our present cytological knowledge I do not believe one would be justified in holding a view that there are chromatin granules of paternal and of maternal origin which are homologous in form and function. It would be desirable to have further evidence on the exact significance and the precise method of synapsis, and an explanation of its apparent absence, but no evidence can be offered from the coelenterates I have studied. It may be added that in Tubularia crocea there was clearly a stage of synizesis (G. T. Hargitt, '09, figs. 30 to 33).

The growth of the egg of Clava is much like that of Campanularia, the food coming from the adjacent enteric cavity; there is no absorption of degenerating oocytes as in Pennaria, Tubularia and others. Growth begins, as shown by figure 4, before the chromatin loops have formed a reticulum, and this may continue for a time, as figure 5 illustrates. At about this period were found particles of chromatin in the cytoplasm (figs. 5 and 6) in the form of granules close to the nuclear membrane. Coincident with further growth, more abundant chromatin granules appear in the cytoplasm (figs. 7 to 9), and with the appearance of these granules yolk formation takes place. There appears to be some close relation between the passing of chromatin from the nucleus into the cytoplam and the marked growth of the egg, and especially with the formation of yolk. I am inclined to interpret this as a causal relation, and think of the chromatin functioning as an enzyme to elaborate (or break down, as the need may demand) food in the cytoplasm. The early growth before emission occurs is due to the digested food of the enteric cavity furnishing the substance and energy. This would mean that the food in the enteric cavity is in a condition to be absorbed directly by the egg and suffices for carrying on the ordinary functions; but for the elaborating of reserve stuffs further changes are essential and these changes are determined and conditioned by the substances released from the nucleus.

C. W. Hargitt ('06) calls attention to the appearance in the living eggs of Clava "at a certain stage of development of a delicate, bluish, pigment, which gradually accumulated in amount

as the egg approached maturity." This pigment was found to show first about the nucleus and later to spread throughout the egg; the pigmentation was correlated with yolk formation in some way. The present study has demonstrated the liberation of substances from the nucleus and has established the fact that this is coincident with the formation of yolk. This is in exact harmony with, and explanatory of, the observations on the formation of yolk in the living egg. Such an agreement of living behavior with inferences from cytological investigations would seem to warrant the conclusion that the morphological structures found in sections are in no measure artifacts, but are normal. Also the conclusion is rendered undoubted that such structures (referred to as chromidia, extra-nuclear chromatin, etc.) are in reality of nuclear origin. This latter point has been doubted by some and recently Beckwith ('14) has said that such particles are not chromatin and do not come from the nucleus but arise in the cytoplasm *de novo*. This point I shall discuss more fully later, but here I desire to call attention to the confirmation from living eggs of the nuclear origin of substances which condition yolk formation within the egg.

The conditions are then essentially alike in the campanularian hydroid, Campanularia, and the tubularian hydroid, Clava. There is the difference that in the former the nucleolus played an important part and in Clava this is apparently not the case. The only indication of activity in the nucleolus of Clava is in the vacuolation (figs. 5 to 9); there is none of the subdivision and gradual dissolution so characteristic of Campanularia. Correlated with this difference in behavior is the fact that the nucleolus of Campanularia contains much chromatin, while in Clava it appears to be a real plasmasome, almost if not entirely devoid of chromatin. A still further correlation lies in the presence of an extremely delicate, fine meshed, faintly staining reticulum in the nucleus of Campanularia, and a deeper staining, coarsely granular and wide meshed reticulum in Clava. The chromatin of the nucleus appears to be the dynamic center and, if this be located in the nucleolus, the latter may function actively, but if the nucleolus be devoid of chromatin, it seems to have a

small part to play in the activities of the cell, and the increased amount of chromatin thereby present in the reticulum renders the latter more obvious.

During the growth of the egg the nucleolus increases enormously in size (fig. 7) though this may be only apparent and due to its vacuolation, but toward the end of the growth period of the egg, the nucleolus decreases in size and finally disappears within the nucleus. I did not find any evidence of bodily migration of the nucleolus into the cytoplasm such as was described by Harm and Hargitt.

During this same period the chromatin begins to form strands (figs. 8 and 9) which condense to produce the chromosomes of the maturation spindle (figs. 10 and 11). Two polar bodies are formed by mitosis as Beckwith shows. In the eggs which show the formation of the polar bodies I found in each case 12 chromosomes, which represents the reduced number. Beckwith in her figures of the maturation spindles of Clava shows 10 chromosomes, but it is not indicated whether this is meant to indicate accurately the entire number. I have carefully examined both maturation spindles and polar bodies, and where ever a count is possible, 12 is the invariable number (figs. 10 and 11 b). The spermatozoon enters the egg of Clava before the maturation has been completed, but the exact time and the place where it enters were not determined. Figure 11 a is an oblique section of the second maturation spindle (the chromosomes not all present in this section) and 11 b (another section of the same egg) shows the first polar body and the sperm nucleus as a vesicular body close to the surface of the egg near the polar body. The stages of the conjugation of the nuclei and the cleavage of the egg were not followed.

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All figures drawn with the aid of the camera lucida   The magnification indicated is the initial magnification, the figures as they appear here have been reduced to $\frac{3}{4}$ original size.

ent).—entoderm. All figures $\times$ 1700.

1   Primitive egg cell in the ectoderm.

2   Primitive egg cells migrating through the supporting layer into the entoderm, from the ectoderm.

3 and 4   Primitive egg cells in the entoderm of the polyp.

5   Growing egg cells in the entoderm of the pedicel of the gonophore. The nuclear reticulum coarsely granular and wide meshed. Chromatin emission starting.
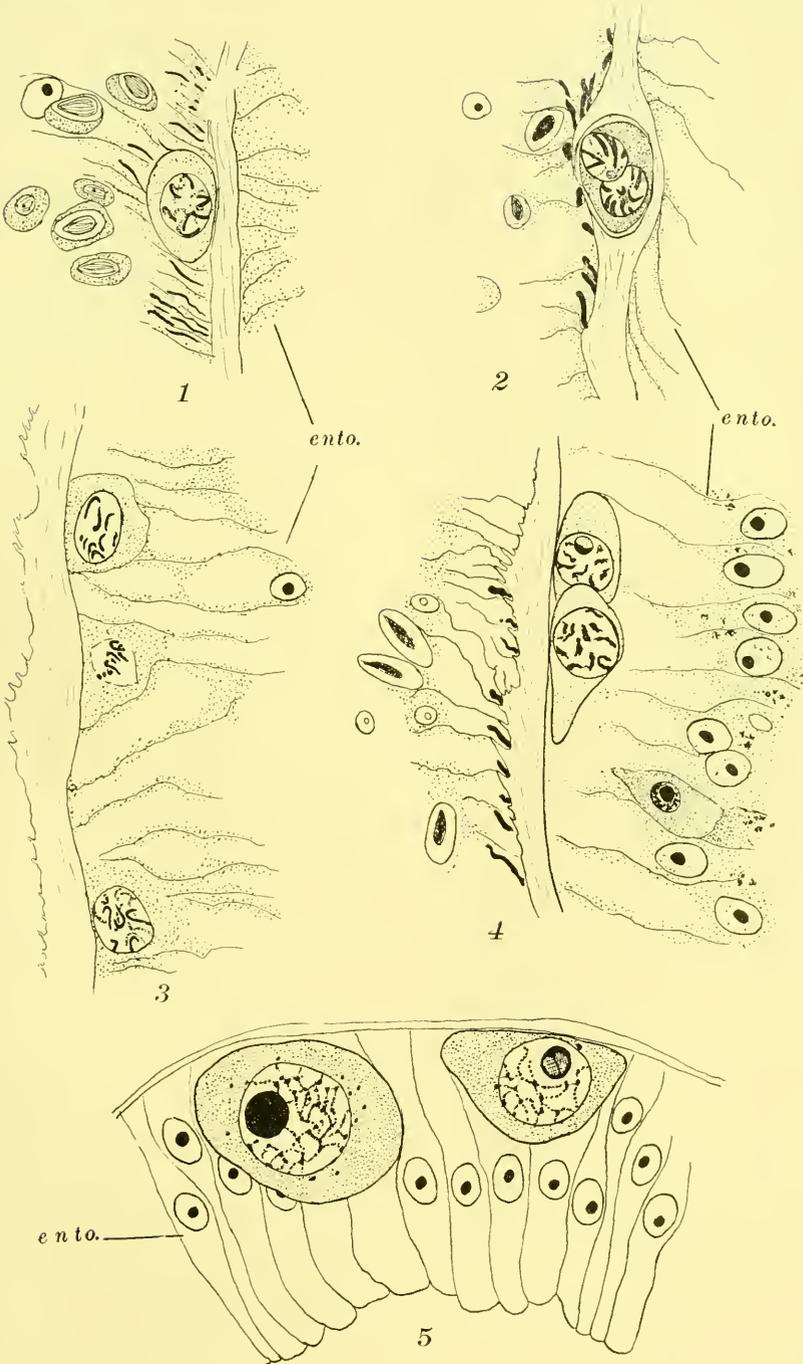
Clava leptostyla

6 Growing egg in position at the end of the gonophore. Chromatin emission.
× 1700.

7 Germinal vesicle of a large growing egg. Nucleolus vacuolated and lightly
staining. Chromatin emission going on, yolk bodies forming. × 1500.

8 Germinal vesicle in the egg at the end of the growth period. Chromatin
emission still continues. Chromatin of the reticulum forming strands which
later become the chromosomes. × 1500.

9 Chromosomes forming in the germinal vesicle, the chromatin emission not
concluded. × 1500.

10 Second maturation spindle with 12 chromosomes; first polar body showing.

11 a, b Two adjacent sections, showing in b the first polar body and sperm
nucleus; in a a portion of the second maturation spindle which contained 12
chromosomes.
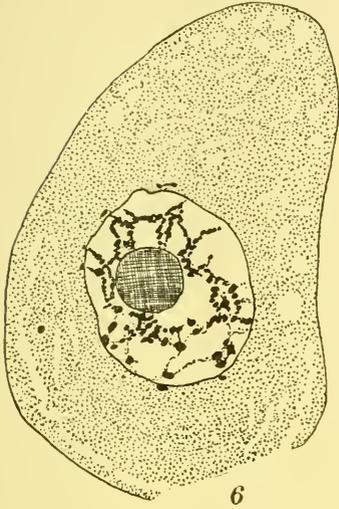
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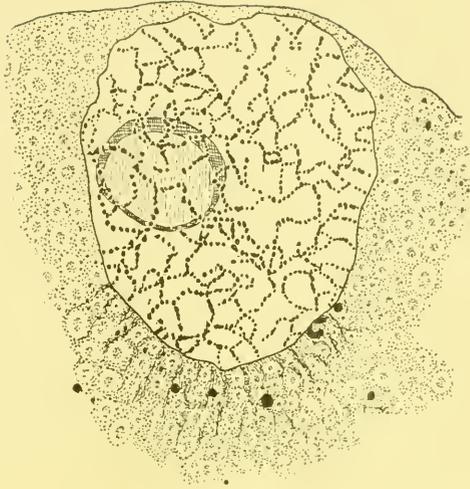
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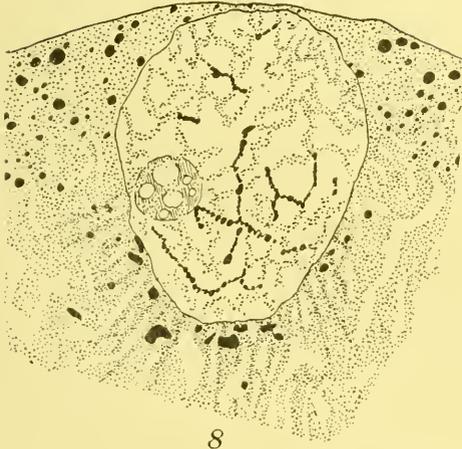
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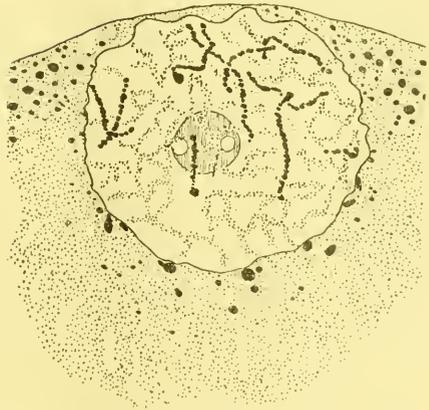
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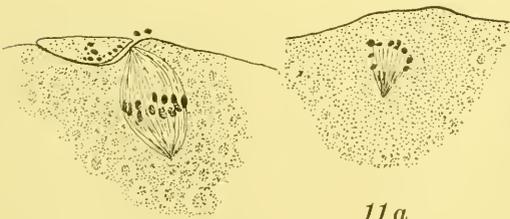
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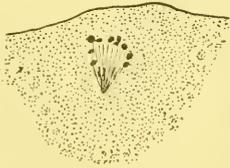
11 a
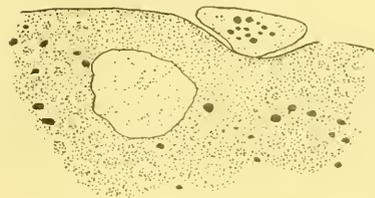
11 b

THE SO-CALLED MANDIBULAR ARTERY AND THE PERSISTING REMNANT OF THE MANDIBULAR AORTIC ARCH IN THE ADULT SELACHIAN

EDWARD PHELPS ALLIS, JR.

Menton, France

TWO FIGURES

In connection with my recent work (Allis, '15) on "the homologies of the hyomandibula of the gnathostome fishes" I frequently had to consult drawings of dissections, made during the summer of 1914 by my assistant, Mr. John Henry, of the adult Heptanchus cinereus and the adult Mustelus (probably vulgaris). These dissections had been made with special reference to a study of the ligaments related to the mandibular and hyal arches, and I particularly wished to know the relations of these ligaments to the so-called afferent mandibular artery of my several works on the Selachii. This latter artery I considered to be the persisting primitive aortic vessel of the mandibular arch, and it was to be traced upward, if possible, to its assumed connection with the definitive afferent pseudobranchial artery, this connection not having been found in any of the dissections made in connection with my earlier works relating to this subject (Allis, '11 b, '12 b).

In Heptanchus this connection was, again, not found, but it was found in Mustelus, and I noticed that, in the drawings relating to this latter fish, the nervus mandibularis internus facialis ran downward and forward, external to the so-called afferent mandibular artery. That a branch of the posttrematic nerve of the hyal arch, a branch distributed to the inner surface of the mandible, could lie external to the primitive aortic vessel of the mandibular arch seemed, in itself, highly improbable. Furthermore, I had several years before, in Amia (Allis, '00, p. 114), found this nerve lying internal to the so-called arteria hyoidea,

which I considered to be the persisting primitive aortic vessel
of the mandibular arch of that fish and hence the homologue
of the so-called afferent mandibular artery of my later descriptions
of the Selachii.   There was thus evidently some error of obser-
vation somewhere in these several works, or actually differing
conditions which needed explanation.

Mr. Henry's drawings and dissections were first controlled,
and, as they were found to be correct, he was instructed to control
the relations of the artery to the nerve in Amia; where he found
them, both in embryos and the adult, as given in my earlier work.
He then also found the same relations of artery to nerve in young
specimens of Cottus aspera that had been sectioned, in the adult
of Scorpaena scrofa, and in an 80 mm. specimen of Lepidosteus.
It was thus probable that in both embryos and adults of all the
Teleostei and Holostei the so-called arteria hyoidea lay external
to the nervus mandibularis internus facialis, as it is evident that
the primitive aortic vessel of the mandibular arch naturally
should.   Sections of 36 mm. and 55 mm. embryos of Mustelus
vulgaris were then examined, and there also the artery was found
to lie external to the nerve.   It accordingly seemed quite certain
that the so-called afferent mandibular artery of my several
descriptions of the adult selachian could not be the persisting
primitive aortic vessel of the mandibular arch; for that this
arterial vessel of Mustelus could have changed its relations to
an important branch of the nervus facialis, from external to
internal, after passing the age represented by a 55 mm. embryo,
I considered wholly improbable.

In my work on Chlamydoselachus I had found a long but deli-
cate branch of the so-called afferent mandibular artery which
lay external to and approximately parallel to the latter artery.
Its relations to the nerves and muscles of the region, which I
at the time considered of no great morphological significance,
had not been noted, but the artery was said (Allis, '11 b, p. 516)
to run upward in the hyal arch, to supply the muscles of the
region, and apparently to represent the anterior efferent
artery of the hyal arch.   In Mustelus, Mr. Henry had also found
a very small artery which had an approximately similar course.

This artery he had designated, in his drawings, as an external branch of the afferent mandibular artery, and it was shown lying external both to the nervus mandibularis internus facialis and to the intermandibularis portion of the superficial constrictor of the hyal arch.

This small and apparently unimportant branch of the so-called afferent mandibular artery of these two fishes might accordingly be the persisting remnant of the primitive mandibular aortic vessel. I accordingly decided to have these arteries again traced in the adult Chlamydoselachus, and the relations of the arteries to the nerves, ligaments and muscles of the region were now to be carefully determined. This dissection was confided to my assistant Mr. Jujiro Nomura, and, as the specimen used had to be preserved for other work, the arteries were not injected with ink as they had been in the earlier dissections. This makes the dissection much longer and more difficult, but is perhaps not without certain compensating advantages. The veins, unfortunately, could not be satisfactorily traced and are left out of consideration. The arteries, as now found in this one specimen of Chlamydoselachus, are shown in the two accompanying figures, but before describing them brief reference must be made to certain of the earlier descriptions of these vessels.

Dohrn ('85, '86), as is well known, found two efferent arteries developed in each of the branchial arches of selachian embryos, and those two arteries are said to persist in the adult, one lying posterior to the branchial rays and the other anterior to them. In the hyal arch of these embryos only the posterior efferent artery is developed, the anterior one being represented by lacunae which are said to be distributed along the anterior surface of the arch, but not to unite to form a continuous vessel. In the mandibular arch neither posterior nor anterior efferent arteries are developed, the primitive aortic vessel, arising, ventrally, either directly from the truncus arteriosus or from the ventral end of the afferent artery of the hyal arch, there forming both the afferent and the efferent vessels of the arch A posterior efferent artery was, however, considered by Dohrn ('86, p.

147) to have primarily extended the full length of this arch; and if the mouth were developed from a pair of gill slits, as Dohrn maintained, there would seem no reason why an anterior efferent artery should not also have extended the full length of the arch.

In the hyal arch certain of the lacunae that, in embryos, represent the undeveloped anterior efferent artery are said by Dohrn to become connected with the posterior efferent artery by a cross-commissural vessel similar to the one that connects the corresponding arteries, at the middle of their lengths, in each of the branchial arches, and this hyal commissure is said to be prolonged anteriorly and to fall into the primitive aortic vessel of the mandibular arch. A hyo-mandibular cross-commissure is thus formed, connecting an efferent hyal artery with an afferent mandibular one, and it becomes the basal portion of the secondary and definitive afferent artery of the spiracular gill. That portion of the primitive mandibular aortic vessel which lies ventral to the point where it is joined by this cross-commissure, is first called, by Dohrn, the arteria thyreoidea, but, as it is said to either receive or give off, at its dorsal end, an artery that is distributed to the lower jaw, Dohrn later calls it the arteria thyreo-mandibularis. Still later, because this artery, in embryos, still carries blood to the spiracular gill, it is called the arteria thyreo-spiracularis; but as this term implies the inclusion of the arteria spiracularis of Dohrn's descriptions, the term arteria thyreo-mandibularis will be here employed.

This arteria thyreo-mandibularis of embryos is said by Dohrn ('85, pp. 6–7) to lie anterior to a branch of the nervus facialis, and to be separated from the efferent artery of the hyal arch by a part of the corresponding (entsprechenden) hyal muscles; and, as it is said to run upward behind the mandibular cartilage and to have been theretofore called the arteria mandibularis (wo sie bisher erwähnt ward, hiess sie A. mandibularis), there seems no reason to doubt that Dohrn considered this artery of embryos to be identical with the so-called arteria mandibularis of the then existing descriptions of the adult fish. This arteria thyreo-mandibularis is said by Dohrn to become relatively smaller and less important, the older the embryo, not only in the Selachii

but also in certain of the Batoidei, and Raffaele ('92, p. 46) says that it aborts and completely disappears in the Torpedinidae. Raffaele further says that the posterior efferent arteries of the branchial arches develop, in Torpedo, before the anterior ones, this order of origin thus accounting for the presence of a posterior artery and the absence of an anterior one in the hyal arch of the embryos examined both by himself and by Dohrn. In the branchial arches, both of the Selachii and the Batoidei, the anterior efferent artery is said by Dohrn later to become the larger and more important one, and, in the Holocephali and Teleostei, Parker ('86) says that this anterior efferent artery alone is found in the adult.

In embryos of Mustelus, probably somewhat older than the embryos studied by either Dohrn or Raffaele, Wright ('85) found no afferent mandibular artery arising either from the truncus arteriosus or the ventral end of the afferent artery of the hyal arch, but he did find an artery, apparently not described by Dohrn, that had its origin from the ventral end of the anterior efferent artery of the first branchial arch. This artery is said by Wright first to send a branch to the thyreoid gland and then to run

upwards and outwards on the ventral surface of the hyoid arch, supplying the parts between it and the mandible, till it reaches the hyomandibulo-hyoid articulation, and probably anastomoses there with the afferent artery of the mandibular pseudobranch. From its course I conclude that this artery is the thyro-mandibular artery of Dohrn; if so, its newly acquired origin from an efferent vessel of the first branchial arch is worthy of note. We shall find that it corresponds entirely in its course to a vessel which originates in the same way in Lepidosteus, and which is obviously the art. hyoidea or hyo-opercularis of the Teleostei.

Later in the same work Wright says of this artery in Lepidosteus:

As remarked above, its course agrees with that of the thyro-mandibular artery of Dohrn. It appears to me to be homodynamous with the nutritive or branchial arteries which spring from the succeeding efferent arteries, . . . The Selachians also possess similar nutritive vessels, and it is easy to understand why that of the hyoid arch should be larger than those of the succeeding arches, whereas it is diffi-

cult to reconcile Dohrn's account of the origin of the thyro-mandibular artery with the conditions in the stage of Mustelus above described. It is difficult to conceive an aortic arch losing its connection with the truncus arteriosus, and becoming connected with the efferent vessel of the second arch behind it.

From the above quotations it would seem that Wright considered the artery described by him in Mustelus to be not only the homologue of the arteria hyoidea of Lepidosteus and the Teleostei, but also to be identical with the arteria thyreo-mandibularis described by Dohrn in other selachian embryos, and that, rather than question this identity, he was inclined to doubt the origin of the artery, as described by Dohrn, from the afferent hyal artery. Dohrn however later ('86, pp. 166–168) found that the mandibular aortic arch of trout embryos, which he identifies as the so-called arteria hyoidea of the adult teleost, has its point of origin secondarily transferred from the truncus arteriosus to the ventral end of the efferent artery of the first branchial arch, these two connections, in certain embryos, even both existing, cotemporaneously, before the one with the truncus arteriosus definitely aborts. The so-called arteria hyoidea of teleosts he identifies as the homologue of the arteria thyreo-mandibularis of his own descriptions of selachian embryos, and, as he particularly discusses Wright's work above referred to and says nothing to the contrary, he certainly considered the artery described by that author in Mustelus to be identical with the arteria thyreo-mandibularis of his own descriptions.

In the adult selachian, the earliest descriptions that I can find of the artery here under consideration, in the works at my disposal, is that by Parker ('86) of Mustelus antarcticus. In Balfour's ('81) still earlier works a mandibular artery is frequently referred to, but the references would seem to be to the artery in embryos only. Parker (l. c.), who evidently had not seen either of the works by Dohrn and Wright to which I have above referred, says that the 'mandibular artery' of Mustelus antarcticus arises from the ventral end of the 'first efferent branchial artery,' the efferent artery thus referred to being the posterior efferent artery of the hyal arch. The mandibular artery is then said to

run upward along the outer face of the hyal arch immediately internal to the 'ventral border of the mandible'; and Parker adds:

From the analogy of Callorhynchus it would seem, therefore, that the mandibular artery is to be looked upon as a part of the original mandibular aortic arch, the rest of the ventral portion of which, represented by the pseudobranchial artery, has acquired a secondary connection, comparable to the transverse commissures, with the first efferent branchial [posterior efferent hyal] artery.

In all these earlier descriptions and conclusions there is thus nothing to make one not previously so disposed even suspect that the so-called mandibular artery, or arteria mandibularis, of the adult selachian is not the persisting ventral, subcommissural portion of the aortic vessel of the mandibular arch together with that branch of that aortic vessel which is said by Dohrn to be distributed, in embryos, to the lower jaw. I have accordingly so considered it in all my earlier works, and, seeking to avoid confusion, have called the basal portion of the artery the afferent mandibular artery, limiting the term mandibular artery, as in current descriptions of the Teleostei, to the branch sent to the lower jaw. I however suggested, in one of my earlier works (Allis, '12 a, p. 135), that the origin of the persisting afferent mandibular artery of the Teleostei from an anterior prolongation of the lateral hypobranchial artery would suggest that it 'might be primarily an efferent and not an afferent, or aortic, vessel.'

The conditions in the adult Chlamydoselachus, as shown in the accompanying figures, may now be described.

In the present specimen of Chlamydoselachus, as in the one described in my earlier work, the lateral hypobranchial artery (ventral lateral longitudinal commissure of the efferent branchial arteries) continues forward beyond the posterior efferent artery of the hyal arch, and, crossing the ventral surface of the ceratohyal, immediately gives off two branches, one of which runs downward along the anterior edge of the ceratohyal and the other upward along the same edge. The former goes to the thyroid gland and is the arteria thyreoidea properly so-called. The other (*aehy*, fig. 2) runs upward between the ceratohyal and
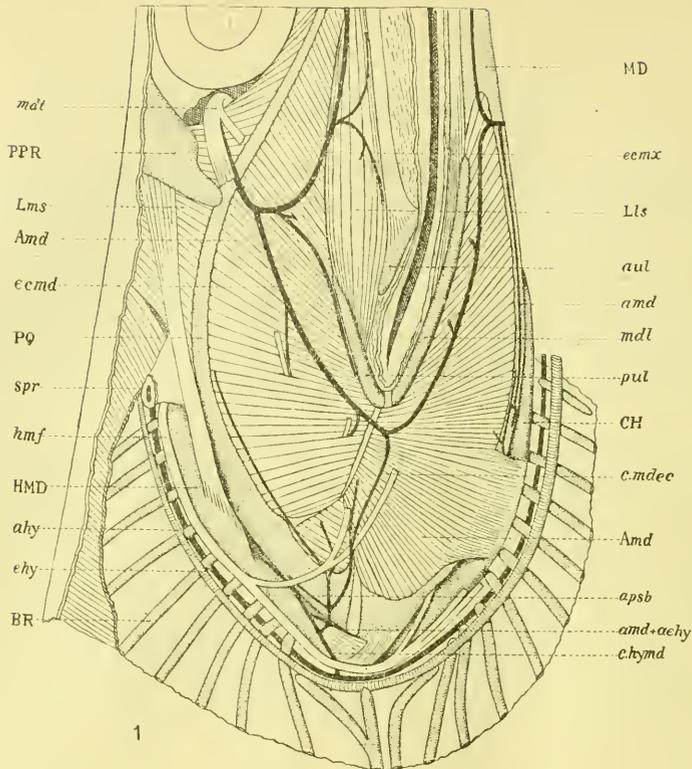
INDEX LETTERS

a., branch of anterior efferent hyal artery to angle of gape of mouth

aal., afferent artery of first branchial arch

aul., anterior upper labial cartilage

aehy., anterior efferent hyal artery

ahy., afferent hyal artery

Amd., musculus adductor mandibulae

amd., afferent mandibular artery

amd.+aehy., artery formed by fusion of afferent mandibular and anterior efferent hyal arteries

apsb., afferent pseudobranchial artery

ath., thyroid artery

BR., branchial rays

CH., ceratohyal

c.hy-md., commissural vessel between the efferent hyal and afferent mandibular arteries

c.md-ec., commissural vessel between the afferent mandibular and external carotid arteries

ecmd., mandibular branch of external carotid artery

ecmx., maxillary branch of external carotid artery

ehy., efferent hyal artery

HMD., hyomandibula

hmf., truncus hyoideo-mandibularis facialis

lhbr., lateral hypobranchial artery

Lls., musculus levator labii superioris

lmh., ligamentum mandibulo-hyoideum

Lms., musculus levator maxillae superioris

MD., mandibula

mdl., mandibular labial cartilage

mdt., nervus mandibularis trigemini

mhbr., median hypobranchial artery

mif., nervus mandibularis internus facialis

pul., posterior upper labial cartilage

pehy., posterior efferent hyal artery

PPR., postorbital process of chondrocranium

PQ., palatoquadrate

sm., submental artery

spr., spiracle

ta., truncus arteriosus

th., thyreoid gland

MD

sm

lhbr

amd

ecmd

th

ath

lhbr

lhbr

mdt

Amd

mdt
lhbr

aehy

CH

pehy

amd

a

lmh

mif

ama+aehy

ahy

mhbr

aal

ta

2
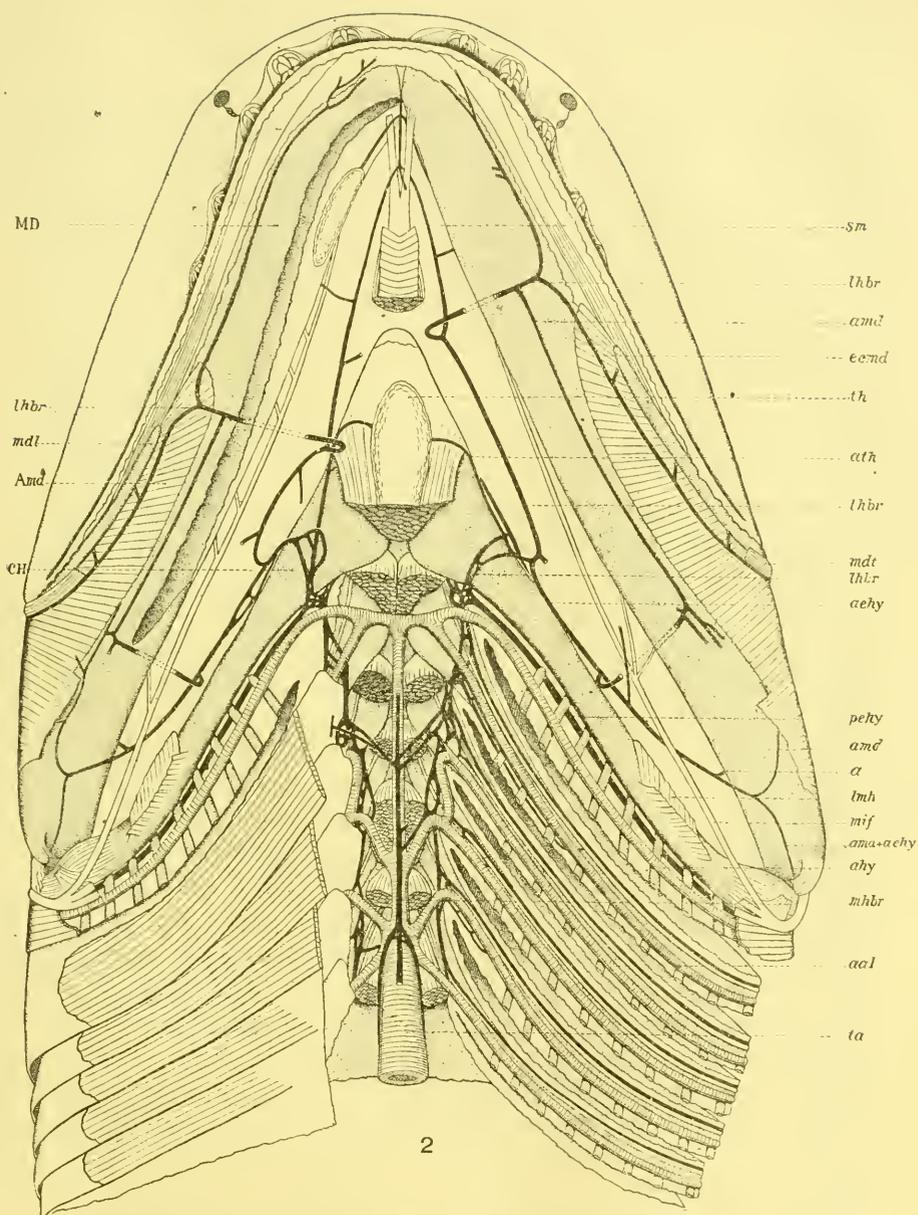
Fig. 1 Lateral view of the head of Chlamydoselachus, the muscles related to the hyal arch removed so as to expose the arteries and the branchial rays.

Fig. 2 Ventral view of the same, the muscles removed and the mandibles pulled somewhat apart so as to show the nerves and arteries. Ventral edge of mandibular cartilage of right side (left in figure) slightly cut away.

the mandibula, and, from its course and distribution, is unquestionably the mandibular artery (arteria mandibularis), not only of Parker's descriptions of the adult Mustelus antarcticus, but also of the similarly named artery in Carazzi's ('05) descriptions of the several selachians examined by him. It will accordingly be so called for the present, although it will be later shown that it is the anterior efferent artery of the hyal arch.

Having given off these two branches, the lateral hypobranchial artery continues anteriorly, internal to the musculus intermandibularis, and, opposite the thyreoid gland on one side of the head of my specimen (but slightly anterior to that gland on the other), sends a branch forward internal to the mandibula. The artery itself then turns outward around the ventral edge of the mandibula, and, piercing the musculus intermandibularis near its lateral edge, reaches the external, ventro-lateral surface of the mandibula. The branch sent forward internal to the mandibula lies internal to the musculus intermandibularis, and is the submental artery of Parker's descriptions of Mustelus antarcticus. Slightly posterior to the symphysis of the mandibles it unites, in the median line, with its fellow of the opposite side, and a small median branch is sent posteriorly and a somewhat larger one anteriorly. From this latter artery, at the symphysis, a branch is sent postero-laterally, on either side, along the internal surface of the mandibula, near its ventral edge, and is lost in a mass of tissue which looks like degenerate glandular tissue.

The submental artery, although it actually runs anteriorly, is morphologically a ventral branch of the lateral hypobranchial artery directed toward the distal end of the mandibula. It is accordingly a serial homologue of the more posterior ventral branches of the lateral hypobranchial artery, one of which branches goes to the thyreoid gland while others unite in the median line to form the median hypobranchial and coronary arteries (Allis, '11 b).

The lateral hypobranchial artery, having reached the ventro-lateral surface of the mandibula, sends a delicate branch dorso-posteriorly, immediately lateral to the lateral edge of the musculus intermandibularis and, itself continuing onward, falls

into what I described, in my earlier work, as the mandibular branch of the arteria carotis externa. This mandibular branch of the carotis externa is apparently the buccal artery of Parker's descriptions of Mustelus antarcticus. It accompanies the nervus mandibularis trigemini and is prolonged, beyond the point where it is joined by the lateral hypobranchial artery, toward the tip of the mandible. This terminal portion of the mandibular branch of the carotis externa is thus, in its relations to the lateral hyopobranchial artery, a serial homologue of the more posterior ventral branches of that artery.

That branch of the lateral hypobranchial artery which is sent dorso-posteriorly before the hypobranchial artery falls into the carotis externa has a course approximately parallel to the so-called arteria mandibularis. It lies slightly anterior to that branch of the nervus mandibularis externus facialis which supplies the hyomandibular line of latero-sensory organs, lies external to the nervus mandibularis internus facialis, and is separated from the hyal arteries by both the musculus intermandibularis and the musculus interhyoideus. At about the posterior quarter of the length of the mandibula it receives a commissural branch from the arteria mandibularis and then itself unites with that artery, having first passed internal to, or perforated, the tendon of a superficial bundle of the musculus adductor mandibulae which crosses from the mandibular to the hyal arches (Fürbringer, '03). This small and relatively unimportant artery thus has all the relations to the cartilages, nerves and muscles of the region that a persisting remnant of the primitive aortic vessel of the mandibular arch should have, according to Dohrn's descriptions of that vessel in embryos, and, as it is certainly that artery (if the artery actually persists in the adult), it can be at once called the afferent mandibular artery in order to distinguish it from the so-called arteria mandibularis.

The arteria mandibularis, running upward beween the ceratohyal and mandibula, lies internal to the musculus intermandibularis but anterior, and hence external, to the musculus interhyoideus and constrictor muscle of the hyal arch. In its course it passes internal to both the nervus mandibularis internus facialis

and the ligamentum hyoideo-mandibulare externum of Goodey's
('10) descriptions of this fish, this ligament of Chlamydoselachus
apparently being the homologue of the ligamentum mandibulo-
hyoideum of my descriptions of Amia (Allis, '97). Antero-ventral
to this ligament the artery sends a commissural branch outward
around the ventral edge of the mandibula and then through the
lateral edge of the musculus intermandibularis, where it falls into
the afferent mandibular artery just above described. Postero-
dorsal to the ligament a branch (a, fig. 2) is sent dorso-anteriorly
across the internal surface of the mandibula toward the angle
of the gape of the mouth, where it separates into two portions;
a small ventral one which runs forward along the dorsal edge
of the internal surface of the lower jaw and a much larger dorsal
one which runs forward along the ventral edge of the internal
surface of the upper jaw. This latter branch, on both sides of
the head of this specimen, passes immediately anterior to a rel-
atively deep tubular pocket, or recess, of the lining membrane
of the mouth cavity which, beginning slightly posterior to the
angle of the gape, extends dorso-posteriorly toward the quad-
rato-mandibular articulation. This pocket lies along the exter-
nal surface of the hind end of the palatoquadrate, between that
cartilage and those fibers of the musculus adductor mandibulae
that pass uninteruptedly from the upper to the lower jaw.
Posteriorly it ends blindly, its blind end being attached to liga-
mentous tissues which, continuing on in the line prolonged of the
pocket, are attached to the hind (distal) end of the palatoquad-
rate. The pocket thus lies morphologically anterior to the
palatoquadrate, in the relation to that cartilage that a persisting
remnant either of the mandibular cleft or of a premandibular
cleft would have, and its position, posterior to the musculus
adductor mandibulae, is not unfavorable to its being a remnant
of either of those clefts, for the adductor muscle, if it be derived
from the superficial constrictor of the mandibular arch, could
readily, when it slipped from the external (actually posterior)
edge of the arch on to its anterior (actually lateral) surface,
have acquired a position superficial, and hence morphologically

anterior, to the pocket. A branch of the artery is sent posteriorly, on either side of the pocket, to the adductor muscle.

After giving off this branch to the angle of the gape, the arteria mandibularis becomes much reduced in caliber and soon joins, or is joined by, the afferent mandibular artery. The single artery so formed, continuing onward, passes internal to the 'joint ligament' of Goodey's descriptions, which extends from the articulating ends of the mandibula and palatoquadrate to the corresponding ends of the hyomandibula and ceratohyal, and is then joined by the hyo-mandibular cross-commissural vessel. The artery formed by the union of these three vessels represents that portion of the primitive mandibular aortic arch of embryos which lies dorsal to the hyo-mandibular cross-commissural vessel and which is called by Dohrn the arteria spiracularis. It and the hyo-mandibular cross-commissural vessel together form the so-called definitive afferent pseudobranchial artery of the adult; and, continuing onward, it supplies the spiracular gill. At or near the point where the three vessels unite to form the arteria spiracularis a variable number of branches are given off. One of these branches runs dorso-anteriorly a certain distance along the lateral surface of the dorso-posterior edge of the palatoquadrate. Another branch runs forward along the external surface of the musculus adductor mandibulae and falls into the mandibular branch of the arteria carotis externa. This mandibular branch of the carotis externa is thus connected with the afferent mandibular artery by two vessels, each of which is apparently an anterior prolongation of a commissural vessel connecting the latter artery with the posterior efferent artery of the hyal arch.

In my earlier ('11 b) descriptions and figures of the arteries in this fish, the thyreoid, submental, mandibular and afferent mandibular arteries as now described and identified are all shown, as is also that branch of the mandibular artery (there called the afferent mandibular artery) which is sent to the angle of the gape, there to separate into maxillary and mandibular portions. But the anterior prolongation of the lateral hypobranchial artery beyond the afferent mandibular artery as at

present identified, the dorsal connection of this latter artery and
the arteria mandibularis with the hyo-mandibular cross-com-
missure, and the prolongation of this latter commissure, ante-
riorly, to fall into the mandibular branch of the carotis externa
were not then found and are not shown. In Mustelus and Hep-
tanchus I now also find these several arteries in what would seem
to be strictly equivalent conditions, but as the dissections of
these fishes were not made primarily to determine the minor
arterial connections, certain of the commissural vessels were not
found. The afferent mandibular artery, in both Mustelus and
Heptanchus, is also a much less important vessel than in Chlamy-
doselachus, and there are apparently also certain minor differ-
ences in the distribution of the arteries. It is however certain,
from the conditions found in these three fishes, that the so-called
mandibular artery, or arteria mandibularis, of current descrip-
tions of the adult selachian is not the persisting mandibular
aortic arch, which it is primarily the purpose of this investi-
gation to establish, and the relations of this artery to the cerato-
hyal, to the nervus mandibularis internus facialis, and to the
muscles of the region all indicate that it is, as already stated,
the anterior efferent artery of the hyal arch.

This anterior efferent hyal artery must then be developed, in
stages later than those examined by Dohrn, from the lacunae
said by that author to be distributed along the anterior surface
of the hyal arch of embryos, the primitive aortic vessel of the
mandibular arch then diminishing in relative size, or, as is said
to be the case in Torpedo, completely aborting. That this ante-
rior efferent hyal artery should develop later than the posterior
one, is wholly in accord with Raffaele's statement, above referred
to, that the anterior efferent arteries of the branchial arches
develop later than the posterior ones; and, as this anterior efferent
hyal artery must primarily have fallen into the cross-commissural
vessel from the hyal to the mandibular arches, and as both it and
the artery that I have here considered to be the afferent man-
dibular artery carry aerated blood to the spiracular gill, there
would seem to be nothing singular in the fact that, in Chlamy-
doselachus, they have coalesced in their dorsal portions and there

appear as a single artery. It is however evident that the fusion of these two vessels, their origin ventrally from the lateral hypobranchial artery, and their connection, between these two points, in Chlamydoselachus, by a cross-commissural vessel, all suggest that the so-called afferent mandibular artery may not be a persisting portion of the primitive mandibular aortic vessel, as all embryological investigations would seem to establish, but a posterior efferent, or secondary afferent mandibular artery similar to the secondary afferent arteries that I have described in the hyal arch of Amia and certain teleosts (Allis, '12 a). This would then account for the position of the artery, in Chlamydoselachus, posterior to the 'joint ligament' of Goodey's descriptions; for, if the ligaments connecting the mandibular and hyal arches are developed, as I have recently suggested ('15), from tissues that represent the undeveloped branchial rays of the mandibular arch, the primitive afferent mandibular artery would normally lie anterior, instead of posterior, to the ligament. This must be determined by further embryological study of these vessels, and it is well to bear in mind, in this connection, Kellicott's description of the development of the definitive anterior hyal artery in Ceratodus. He says ('05, p. 202):

So that while no direct observations could be made bearing upon this point, all the circumstances point to the development of a branch from the anterior efferent artery of the first branchial arch upward, forming the (definitive afferent) hyoidean artery. The efferent (epibranchial) hyoidean artery is derived from the hyoidean aortic diverticulum and is the anterior carotid artery. The vessels of the hyoid arch of the adult Ceratodus therefore represent a second set of vessels and are not comparable with the vessels of other forms—particularly the other Dipnoi, where, as already pointed out, the hyoid vessel appears as one of the original afferent branchial series.

The artery that, in Chlamydoselachus, runs forward across the external surface of the musculus adductor mandibulae to fall into the mandibular branch of the carotis externa is apparently, as already stated, a direct anterior prolongation of the cross-commissural vessel from the hyal to the mandibular arches. If that portion of the carotis externa which lies immediately dorsal to the point where it is joined by this cross-commissure

were to abort, so separating the dorsal and ventral portions of
the carotis from each other, the conditions described by Parker
in Mustelus antarcticus would arise, the dorsal portion of the
carotis becoming the buccal artery of that author's descriptions
and the ventral portion becoming the strictly mandibular part
of the mandibular artery of that fish. If, on the contrary, the
cross-commissural vessel of Chlamydoselachus were to abort,
the artery distributed to the mandible would become wholly a
terminal portion of the carotis externa, as I have shown it in
my earlier figures and descriptions of this fish. The origin, in
embryos, of the mandibular branch of the mandibular aortic
arch from the extreme dorsal end of the arteria thyreo-mandib-
ularis, so particularly and carefully described by Dohrn, would
then receive full explanation, and the continuous carotid vessel of
Chlamydoselachus would seem to be the anterior efferent artery
of the mandibular arch, its intimate association with the nervus
mandibularis trigemini seeming to preclude its being a pre-
mandibular artery. If it be the anterior efferent mandibular
artery, the secondary connection, in Amia (Allis, '12 a), of its
dorsal portion with the mandibular pseudobranch is fully ex-
plained, and the several forms of secondary afferent pseudobran-
chial arteries found in the Teleostei could be readily derived from
it. The ventral portion of the artery would, accordingly as it
retained its connection with the dorsal portion or with the
afferent mandibular artery, give rise to the variations found
(or at least described) in these latter fishes in the artery dis-
tributed to the mandible.

The conditions thus found and thus interpreted in selachians
favor the suggestion (Allis, '08 a, p. 107) that the carotis externa
is developed in connection with the prehyal portion of the dorsal
lateral longitudinal commissure of the efferent branchial arteries,
and also that its mandibular branch is formed by the anterior
efferent artery of the mandibular arch. The maxillary branch
of the carotis would then be the anterior continuation of the
dorsal lateral commissure, and from this artery, in Chlamydo-
selachus, a branch is sent ventro-posteriorly along the dorsal edge
of the posterior upper labial cartilage, and another toward the

corresponding edge of the anterior upper labial, both branches suggesting efferent arteries of a premandibular arch.

A further fact that is in favor of the origin of the carotis externa from the dorsal lateral commissure is the course of the carotis through the trigemino-facialis chamber in the Holostei and in many of the Teleostei. This course of this artery has always been to me one of the puzzling facts in the cranial anatomy of fishes. But if that part of the artery which traverses the trigemino-facialis chamber represent a part of the dorsal lateral commissure, and the lateral wall of the trigemino-facialis chamber be derived from the extrabranchial of the mandibular arch, as I have lately sought to establish (Allis, '15), this course of the artery is wholly natural; for in Lamna, the one remaining specimen of an adult selachian that I have which is suitable for this purpose, and which I have just re-examined, I find that the attachment of the extrabranchial of the first branchial arch to the inner cartilages of that arch is lateral to the dorsal lateral commissure of the efferent arteries. When the extrabranchial and pharyngobranchial of the mandibular arch fused with the neurocranium to form, respectively, the lateral wall and the floor of the trigemino-facialis chamber, the dorsal lateral commissure naturally became enclosed in the chamber so formed. And this is, conversely, a further fact in favor of my conclusion that these elements of the mandibular arch have been used to form the walls of the chamber.

The artery sent, in Chlamydoselachus, from the anterior efferent hyal artery to the inner surface of the gape was not found in either Mustelus or Heptanchus, but this does not necessarily mean that it does not there exist, for, as already stated, the dissections of these two fishes were not primarily made with reference to a study of all the arteries of the region. What the significance of this artery is could not be determined, but it is to be noted that, like the nervus mandibularis internus facialis, it is a structure of hyal origin distributed to the inner surface of the mandibular arch, and it apparently has some definite relation to the little pocket in the lining membrane of the mouth cavity. In my work on Polyodon, I found (Allis, '11a, p. 286),

in this same region, a mass of 'dense nervo-vascular' tissue
supplied by a branch of the mandibular branch of the carotis
externa, and I then thought that this tissue suggested "a rem-
nant of glandular tissue of some sort, possibly the remnant of a
portion of the mandibular gill." In Polypterus, Mr. Henry
now also finds, in this same place, a much vasculated tissue
supplied by that mandibular branch of the carotis interna of that
fish that I (Allis, '08 b) considered as probably the persisting
dorsal remnant of the mandibular aortic arch. A further study
of the arteries in this fish now makes it plainly evident that this
branch of the carotis interna is simply the maxillo-mandibular
portion of the carotis externa, the carotis externa of my earlier
descriptions being simply the ophthalmic branch of that artery,
somewhat widely separated from the maxillo-mandibular branch.
I now also find that the mandibular portion of this latter artery
falls, ventrally, into an anterior prolongation of the lateral
hypobranchial artery, exactly as in selachians, and from this
anterior prolongation of the hypobranchial artery, before it
reaches the carotis externa, two delicate branches are given off
which quite unquestionably represent the afferent mandibular
artery and the anterior efferent hyal artery of the present de-
scriptions of Chlamydoselachus. These two delicate arteries
are connected by cross-commissure, as in Chlamydoselachus, and
then, running, one anterior and the other posterior to the nervus
mandibularis internus facialis, they unite as they fall into the
artery described by me in my earlier work as probably the hyo-
mandibular cross-commissural vessel. This identification of
this latter vessel I now find to have been correct. From the point
where it is joined by the two delicate arteries above described,
two branches arise. One of these branches runs forward, internal
to the muscles of the region, and joins the mandibular branch
of the carotis externa, thus corresponding, excepting in its re-
lations to the muscles, to the anterior prolongation of the hyo-
mandibular cross-commissure of Chlamydoselachus. The other
branch runs upward posterior to the spiracular canal and joins
the little branch said by me, in my earlier work, to supply the
thymus. About midway in its course it traverses a much vas-

culated region which strongly suggests a rudiment of a branchial gill. The position of this vascular tissue, internal to the anterior edge of the hyomandibula, is that of the mandibular pseudobranch in Amia and the Teleostei, but its relation to the postspiracular artery above described would indicate that it is a remnant of a hyal gill, and hence probably the disappearing anterior hemibranch of that arch.

Palais de Carnolès, Menton, France
    August 1st, 1915

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A COMPARATIVE STUDY OF THE CHROMOSOMES OF SIX SPECIES OF NOTONECTA[1]

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ONE HUNDRED AND FIFTEEN FIGURES (SEVEN PLATES)

CONTENTS

I. INTRODUCTION

A previous study of the chromosomes of three species of Notonecta (Browne '13) proved of such interest as to lead me to undertake a more comprehensive study of the chromosomes of this genus. It is with the view of making a comparative cytological examination of all the species of this genus that the present paper is written dealing with three additional species. Owing to the fact that certain chromosomes are characteristic in

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size, position and behavior, and also to the fact that the differ-
ences in the chromosome number and grouping are relatively
slight in the different species, Notonecta offers an exceptionally
good field for such a comparative study.   In order to ascertain
the relation between the chromosomes and somatic characters,
one of the chief aims of modern cytology, a comparative cyto-
logical investigation of closely related forms seems a method of
attack which should accompany the experimental method.
Moreover, it would seem that the study of different chromosome
groups within a genus should give a clue to the relationship and
possible evolution of the different species, so that a phylogenetic
tree based on the chromosomes would correspond with one based
on somatic characters and geographical distribution.   A thorough
cytological examination has now been made, at least with regard
to the spermatogenesis, of six out of twenty species of Notonecta.
This comparatively limited study will, of course, not guarantee
any sweeping or final statements as to the relation of particular
chromosomes to particular body characters, or as to the precise
phylogeny of the species; only a few general and tentative sug-
gestions in this regard are offered in this paper.

The problem concerning the chromosomes as giving a clue
to the relationship of different forms is one to which Montgom-
ery ('01, '06) devoted considerable attention.   He argued that
the chromosomes are conservative elements and less modifiable
than the soma, and that they should therefore be considered as
of value in determining the relationship of a particular group.
He was, however, dealing with the Hemiptera in general and
was considering only the numerical relations of the chromosomes.
It is not surprising, therefore, that he found this problem in-
creasingly difficult of solution as his study became more exten-
sive.   A glance at the chromosome numbers of different species
of Hemiptera as listed by him in 1906 will show how difficult
such a task would be.   Had he limited himself to families, an
effort to find a rational explanation of a diversity in number of
chromosomes corresponding with the relationship of the genera,
would in many cases probably have proved fruitless.   It has
been repeatedly shown by the work of Wilson, Boring, Stevens,

Morgan and many others that even within certain genera, there is a discrepancy in the number of chromosomes in the different species entirely disproportional to the differences in body characters; for example, different species of phylloxerans have 6, 8, 12, and 22 chromosomes (Morgan '09). So true is this in the case of certain Homoptera, that Boring ('07), after a study of twenty-two species, concluded that the number of chromosomes is of no significance for grouping species into genera. On the other hand, there are fortunately other groups which are quite favorable for a solution of this problem. The orthopteran family, Acrididae, has engaged the attention of McClung for some time in an effort to discover the relation between chromosomes and somatic characters. The forty genera already examined, have, with a few exceptions, shown the same number of chromosomes, and the constancy in number, size and form of the chromosomes has led McClung ('05, '08, '14) to a belief in their importance in taxonomy. A peculiar association of some of the chromosomes in certain genera and species is believed by him to be directly responsible for the generic or specific somatic characters of those forms. The genus Notonecta, consisting as it does of many species differing from each other slightly, but characteristically, both in somatic characters and in chromosome content, offers another very favorable object for the study of this problem.

II. SPECIES OF NOTONECTA

The genus Notonecta includes, according to the entomologists, Kirkaldy ('97) and Bueno ('05), twenty species, thirteen of which are peculiar to America. One species (N. lutea) is common to America, Europe and Asia; one (N. glauca) is common to Europe, Asia and Africa; one (N. lactitans) is African; one (N. handlirschli) is Australian; and three (N. chinensis, N. montandoni, N. triguttata) are Asiatic. My former paper (Browne '13) dealt with three of the American forms common in the eastern United States, N. undulata, N. irrorata, and N. insulata. The present paper deals with the European N. glauca, and two

species common in the western United States, N. shooterii and
N. indica.[*] The other American species are N. variabilis, N.
uhleri, N. howardii, N. raleighi, N. mexicana, N. montezuma,
N. bifasciata and N. nigra, the two latter ones being peculiar
to South America. The genus Notonecta, together with Buenoa
and the Pleinae belong to the family Notonectidae.

III. BRIEF SUMMARY OF THE CHROMOSOMES OF N. UNDULATA, N. IRRORATA, AND N. INSULATA

For a full and detailed account of the chromosomes of these
species, the reader is referred to my former paper (Browne '13).
I here merely outline the chief features of the chromosome groups
in order to have a basis for reference and comparison with those
of the three other species described in detail in this paper.

In N. undulata the number of chromosomes in the first sper-
matocyte division is 14, consisting of a ring of 12 chromosomes
surrounding two small central ones (fig. 1). In side views, these
central pairs often appear linearly arranged, owing to their
proximity and to the precocious division of one pair (fig. 2). In
the second division the number is reduced by one because of the
late conjugation of X and Y, which are not associated in the
first division. In this division the XY dyad lies in the center
of a ring of 12 chromosomes (figs. 3 and 4). The components of
this pair are considerably unequal in size, and, owing to their
presence, the sister groups which go into the spermatids and
spermatozoa are of two kinds (fig. 5 A, B). The spermatogonial
group contains 26 chromosomes (fig. 6). There are five large
chromosomes in the diploid group, representing two large ones
of the haploid and X which is next in size to these. There are
five small chromosomes, representing the two small central ones
of the first division and Y which is the next smallest after these.

In N. irrorata, the number of chromosomes in the first division
is 13, and there is only one small one within the ring (figs. 7 and
8). In the second division there are 12 chromosomes and the
XY dyad lies in the center of the ring (figs. 9 to 11). The sper-
matogonial number is 24 (fig. 12). There are seven large chro-
mosomes, corresponding to three large ones of the haploid group

and X which is next in size. There are six small chromosomes corresponding to the one in the center in the first division, and two small peripheral ones; Y is one of the next smallest.

In N. insulata there are two types of first division groups, one with 14 chromosomes including two small ones in the center, like N. indulata, and the other type with 13 chromosomes including only one small one in the center, like N. irrorata (figs. 13 to 16). When there is only one small one in the center, the other one is often found attached to the largest chromosome (fig. 16). There are 12 chromosomes in the second division including the XY dyad lying in the center (figs. 17 to 19). It is assumed that the second small chromosome is always fused with the largest one in this division. No diploid groups have been found in which the number could be determined. In the haploid groups, a largest and usually a next largest chromosome are noticeable and another small one in addition to the one (or two) in the center in the first division.

IV. THE CHROMOSOMES OF N. GLAUCA

It has been with particular interest that I have examined the chromosomes of N. glauca because of the previous work of Pantel and Sinéty ('06) on the same species. Their account of the maturation divisions is so very meager that I feel justified in giving my own observations in full. I wish to express my sincere thanks to Professor Lefroy of The Royal College of Science, London, for his kindness in supplying me with the insects last summer, and to Professor Dobell, in whose laboratory the material was prepared.

a. Observations

In N. glauca there are, in the first division, 13 chromosomes, which appear in polar view as a ring of 12 surrounding one small central one (figs. 20 to 22). The most striking feature of this group, apart from the general arrangement, is the presence of an exceptionally large chromosome which appears as a double or compound chromosome. A similar appearance is sometimes

presented by the next largest chromosome (fig. 20), though in the majority of cells this is not the case (figs. 21 and 22). A longitudinal section through the center of the spindle shows the small dyad on the central fibers and the large compound chromosome and the next largest one on the peripheral fibers (fig. 23). Consecutive sections of a spindle show the appearance of the entire group of 13 in side view, including the small central dyad, the large compound chromosome, the next largest, somewhat similar in appearance, and another small one, a trifle larger than the central one (fig. 24 A, B, C). Very frequently, the components of the small central dyad lie on different fibers and are not linearly arranged (fig. 25 B). This has been noted previously in N. irrorata, and likewise occurs in N. shooterii. This peculiarity is probably due to the fact that the components of this dyad do not become associated in the late prophase as the components of the other chromosomes do; even when linearly arranged, the components of this small chromosome are usually considerably separated in the full metaphase. Such a precocious separation of the parts of the small central chromosome (the m-chromosome) seems to be characteristic also of the coreids (Wilson et al.). That the small chromosomes in the center in figure 25 B are the components of one central dyad, and do not represent two small dyads is shown by the fact that these chromosomes are distinctly univalent in contradistinction to the other bivalents, and they are passing to opposite poles; moreover, the complete number of 13 is given, only if these are regarded as one chromosome (fig. 25 A, B). Particular attention has been paid to this question, for if this interpretation be not correct, the explanation that the difference between the 14 chromosome groups of some species and the 13 groups of other species is due to the presence or absence of the second small chromosome in the center, would not stand. However, I am convinced from a very careful study of many spindles in side and polar view, that there can be no question that these are the components of one small central dyad and are not two separate dyads.

The large compound chromosome presents a variety of appearances as seen in side view (figs. 23, 24 C, 25 A and 26). In

general it may be described as consisting of four open U's, with the arms often pulled out transversely, sometimes giving it the appearance of being combined with a smaller chromosome. The darker portions in the drawings represent dark areas seen on the chromosome, probably due to the overlapping of two parts, causing thickenings in these regions. Frequently, when the chromosome is seen in face view, that is, on the top of the spindle instead of at the side, a characteristic double cross is apparent (fig. 27). In polar view, the usual appearance is of two diverging, slightly curved arms or open U's (figs. 20 to 22); this probably represents only half of the entire chromosome, for often a more complex structure is evident, consisting of a system of superimposed open U's (fig. 28). In early anaphase, the chromosome appears somewhat irregular (figs. 29 and 30), but the structure of each sister half is clear in later anaphase (figs. 31 and 32). Each part consists of two slightly curved arms or open U's in contact at the base, and bending away from each other. The peculiarity and irregularity of the chromosome in metaphase is probably due to a difference in the amount of fusion and the arrangement of its component parts. The structure of the next largest chromosome, which I described as sometimes similar in appearance to the largest chromosome in the metaphase, is in the anaphase similar in structure, only the parts are smaller and not quite so curved (figs. 31 and 32). These chromosomes in polar views of the anaphase give approximately the same appearance as in side view (fig. 33); this figure shows the entire group of chromosomes in polar view, similar in grouping and size relations to the polar metaphase. Occasionally the chromatic substance from the large chromosome seems to run down over the spindle fibers in the anaphase, giving the figure a very curious appearance (fig. 34). Other observers have noted a thickening of the spindle fibers where attached to the chromosomes, as though some of the chromatic substance contributed to them. This appearance is usual in Notonecta, as I have represented in the drawings, and it is probably an extreme exaggeration of this tendency that is represented in figure 34.

In the telophase of the first division, which is also the prophase of the second, the chromosomes become somewhat crowded, but it is often possible to distinguish the individual ones. The large compound chromosome retains its structure of the anaphase, and can in most cases be followed with ease at this time, and this is also true of the next largest one (fig. 35); both these chromosomes, probably on account of their bulk, usually lag behind the others in division. When on the second spindle (fig. 36), the line of separation between the two curved bars falls along the equatorial plane. In the case of the largest chromosome, therefore, the line of separation in the second division is already marked out in the metaphase of the first, and is in evidence in all the following stages. A side view of the second division shows a characteristic XY pair in the center of the spindle (figs. 36 and 37 B). Owing to the fact that X and Y divide as separate chromosomes in the first division, and as a single chromosome in the second, the number is reduced by one. Consequently, in serial sections of a complete spindle, there are twelve chromosomes, including the XY dyad in the center, and on the peripheral fibers the large compound chromosome, the next largest similar in appearance, and the two small chromosomes, one of which was in the center in the first division (fig. 37 A, B, C). Frequently, the X and Y chromosomes do not take up a linear arrangement, but lie side by side in the metaphase, on different spindle fibers, a peculiarity found in all the species of Notonecta and comparable with the non-linear arrangement of the components of the small central dyad in the first division; this arrangement seems to occur only in those chromosomes whose components normally conjugate late. An entire spindle in which this arrangement of X and Y occurs, is shown in figure 38 A, B, C. Polar views show the same arrangement and size relations of the chromosomes as side views, and sometimes X and Y lie side by side at the same focus (figs. 39 and 40). Whether the two large chromosomes appear double (fig. 39) or single (fig. 40), depends of course, upon whether both sister elements are in view or only one of them. The difference in the amount of chromatin contained in the large chromosomes and the small

ones is well shown in figure 41, where these four chromosomes happen to lie together.

The inequality in the X and Y chromosomes is quite striking in most cases in N. glauca, but is not so great as in the species previously described. There seems to be some individual variation in this respect, the XY pairs from one individual (fig. 42) being more nearly equal than in another (fig. 43). This variation is more marked in N. shooterii, in connection with which the question is discussed more fully. I have found no case in N. glauca in which I have been unable to detect an inequality. This species resembles Nezara hilaris (Wilson '11) in having a constant but often slight difference in the components of this pair, although this difference is perhaps greater in N. glauca. In anaphase, X and Y pass to opposite poles and also the sister elements of the two large chromosomes which at this time are less characteristic in shape (fig. 44). In the late anaphase (figs. 45 and 46) these chromosomes are noticeable as long, somewhat curved bars, and sometimes the larger one gives the appearance of being double on account of a notch in the center, a remnant possibly of the bend in the original U which was one of the four parts composing the large chromosome. Sister anaphase groups in polar view are practically identical except for the central chromosomes which differ in size, sometimes considerably (fig. 47), sometimes less noticeably (fig. 48). Owing to this fact of course, the spermatids and likewise the spermatozoa will be of two kinds with regard to their chromatin content, bearing the usual relation to sex-production.

The growth of the cells in N. glauca is very slow, as has been noted also by Pantel and Sinéty, and on this account most of the cysts containing spermatogonia and early growth stages are empty at the time that the division stages occur, so that few dividing spermatogonia are to be found in the material used for the maturation divisions. The best one occurring in my material is shown in figure 49, and although the number cannot be counted with certainty, the group shows the structure of the two largest chromosomes which appear as long curved rods. This figure also shows the presence of four small chromosomes

corresponding with the central one and the small peripheral one of the first division. Female diploid groups show 24 chromosomes, including four small ones and six large ones, the third pair of large ones being in all probability the X chromosomes (fig. 50).

b. Comparison with the results of Pantel and Sinéty

Pantel and Sinéty, in their monograph on "Les cellules de la lignée mâle chez le Notonecta glauca," have been concerned chiefly with the growth stages and transformation into the spermatozoon, and have only incidently taken up the maturation divisions. However, their criticism of the chromatic figure as being "d'un type malingre" for the study of these divisions seems to me to be altogether unwarranted, for the chromosomes are large, well differentiated in size, and in general seem exceptionally fine for study. Judging from the few figures that are given of these stages, their material was essentially like my own and was prepared in the same way, and I believe that with further study would have yielded similar results.

In the first division, they count in polar views, sometimes 12, sometimes 13 chromosomes, arranged in a ring with one or two in the center, but they state that they can assign no reason for the discrepancy in number. However, in a part of their paper dealing with abnormalities—here the presence of small deep staining corpuscles—a polar view is given, showing a group of 12 chromosomes surrounding one small one, the grouping and size relations corresponding exactly with my figures. The large chromosome, which is such a striking feature of this species, has received particular attention from these authors. They call it the 'chromosome exceptionelle' and describe it as a massive irregular body giving one the impression of a double cross or a system of double V's, and their figures of it in polar metaphase and side anaphase are quite similar to mine. Although they are convinced that it participates in both divisions, they suggest a possible relationship to an accessory chromosome; they abstain however from giving its exact significance. In the second divi-

sion, they do not state the number of chromosomes, but a figure of a polar view shows 12, including one inside the ring, and the 'chromosome exceptionelle' having a cross shape, the next largest of a similar shape, and two small ones, a group similar to those that I have figured. These authors have apparently overlooked entirely the XY chromosomes, and this may explain their hesitancy in stating the number of chromosomes in the two divisions, which should differ by one with these chromosomes present, and should be the same if they are absent.

V. THE CHROMOSOMES OF N. SHOOTERII

A study of the chromosomes of N. shooterii has shown that the number is the same as in N. undulata, but that the grouping is more like that of N. irrorata and N. glauca.

a. Observations

The complete number of chromosomes in N. shooterii in the first spermatocyte division is 14, consisting of one small central one surrounded by a ring of 13 (fig. 51). It is characteristic of this species that a second small chromosome lies usually just within the peripheral ring, but sometimes a little more toward the center (fig. 52). It does not lie typically close to the central one, as is characteristic of N. undulata. The position of these two small chromosomes is more strikingly seen in side view, one of them appearing on the central fibers, and the other one some little distance away on the peripheral fibers (figs. 53 and 54 B). In the actual sections, the position of the second small chromosome somewhat within the peripheral ring is perfectly evident from the fact that it lies at a different focus from the others in the periphery; this cannot be so well shown in the drawings, although I have attempted to do so by different shading. A somewhat oblique section perhaps brings this out more clearly (fig. 55). It often happens in N. shooterii, as described also for N. irrorata and N. glauca, that the components of the central dyad lie side by side, instead of being linearly arranged (fig. 56). When such a spindle is followed through in consecutive sections,

the other 13 chromosomes are to be found, including the second small one, just within the peripheral ring.

In the anaphases, the components of the small central chromosome always lead in the procession toward the poles. The second small chromosome from within the peripheral ring is to be found directly behind the smallest one (figs. 57 B to 61). It is obvious that the mechanical arrangement more or less necessitates the approach of these chromosomes to the pole before the others that are on the outer fibers, and this may be the only explanation of this fact. However, in many cases, these two small chromosomes seem to be connected by fibers, but it is impossible to be sure of this owing to the delicacy of the structures concerned. In a polar view of the late anaphase, the two smallest chromosomes are often to be found quite close together within the ring of 12 other chromosomes (fig. 61). From the position and behavior of the second small chromosome, I believe that it is homologous with the second small one in the center in N. undulata.

In the second division, a characteristic X Y pair is seen in the center of the spindle, surrounded by a group of 12 chromosomes arranged on the peripheral fibers (fig. 62 A, B, C). Among these may be seen the two small chromosomes of the first division. A polar view shows a ring of 12 chromosomes, including two small ones, surrounding X or Y (figs. 63 and 64). These chromosomes are frequently found side by side in the metaphase as described for N. glauca (fig. 65 A). Figure 67 A, B, shows a polar view of two sister groups of chromosomes which have just separated, these being taken from a rather thick section (7μ), where they were at different levels.

The size relations of X and Y are quite variable, as will be noticed by a glance at the figures. In some cases these chromosomes are almost equal, in others they are quite unequal (figs. 62 to 74). The sister groups, therefore, of the late anaphase, are sometimes quite dissimilar in their chromatin content, with respect to the central chromosome, and sometimes almost identical (figs. 75 to 77). In the spermatogonial groups, there are 26 chromosomes, including five large ones, four of which corre-

spond with the two largest ones of the spermatocytes, and the fifth being X; there are four small ones corresponding with the small central one and the one just within the ring in the first division (figs. 78 and 79). Although X and Y are sometimes almost equal in the haploid groups, I have found no case of equality in the diploid, even from the same individual. The oogonial groups give the same number of chromosomes, 26, but have six large ones instead of five, the sixth large one being the second X chromosome (fig. 80).

b. Size relations of X and Y

The X and Y chromosomes, as was mentioned above, differ considerably in their relative sizes in different cells, and this is true to a certain extent for different individuals. Although in many individuals, the inequality of X and Y is considerable, in others the inequality is less marked, and in some cells of these individuals is not apparent at all. This relative inequality is, however, not constant, for in those individuals where there is a tendency to equality, certain cells are found where the inequality is marked. In figures 68 to 74, there are shown the X Y pairs from seven different individuals, the drawings being made as carefully as possible and the extremes and means of inequality being represented in each case. The inequality is well marked in figure 68, fairly evident in figures 69 and 70, less so in the others. In figures 73 and 74, X and Y are in some instances apparently equal, though in one case in each individual, they are markedly unequal. A variation in the size of these chromosomes has been found also by Wilson ('12), in Oncopeltus, and the percentage of cases of equality and inequality was found to vary widely in different individuals in this instance also.

The variation in the relative size of X and Y is well shown in sister polar views of the anaphase (fgs. 75 to 77). Since these groups go directly into the spermatids and spermatozoa, it is evident that in certain cases (where X and Y are different in size), these will fall into two classes, differing in their chromatin content. In those cases, however, where X and Y are equal (fig. 77), there will be no visible difference. Such groups as

these lead, of course, to those forms, of which there are probably many, where there is never a visible dimorphism in the chromatin content of the spermatids; and yet there is reason to believe that such a dimorphism exists. This important fact has been pointed out by Wilson in considering Oncopeltus, and additional evidence is given by the same phenomenon in N. shooterii. There is absolutely no question in this case of the existence of an X Y pair, as these chromosomes are typical and constant in position and behavior in all the species of the genus thus far examined.

VI. THE CHROMOSOMES OF N. INDICA

N. indica has the same number of chromosomes as N. undulata and N. shooterii, and they are arranged in the first division somewhat like those of N. undulata, but slightly modified.

a. Observations

In N. indica, there are two small chromosomes in the center, as shown by spindles in side view (fig. 81). Owing to the proximity of these two chromosomes and to the fact that one pair divides in advance of the other, they most frequently appear arranged in a linear series (fig. 82). This appearance is common in N. undulata also. Three other very characteristic chromosomes appear in side view, two of these being large compound chromosomes, similar to the largest chromosome of N. glauca, only smaller, and a third chromosome, largest of all which consists of a main body and usually a small piece on either side, looking like a compound chromosome, made up of one large and two very small parts. This chromosome can be traced through from early prophase of the first division to the telophase of the second by its characteristic shape, and is the larger component X of the X Y pair. The double chromosomes and the X chromosome are shown as they lie on the spindle in figures 83 and 84. A complete set of chromosomes as seen in side view gives a total of 14, including X, the two doubles and the two small ones in the center (fig. 85 A, B, C). Polar views showing the complete number 14, the general grouping and the size relations are given in figures 86 to 88. The arrangement seems somewhat

irregular at first sight, but closer examination shows a more or less regular ring of ten chromosomes, with the two smallest ones in the center and two intermediate ones between these and the periphery. This arrangement seems quite constant, but owing to the fact that it is somewhat complex, I can not be sure that it is invariable. A slight shifting of one of the chromosomes from the periphery would serve to confuse the arrangement. In side views, the position of these two ex-centric chromosomes is a little diffcult to determine, except when they happen to lie in the section which includes the two small chromosomes and in a plane determined by the central and outermost chromosomes, as indicated by focusing. The ex-centric chromosomes determined in this way are indicated in light gray in figures 81, 82 and 85 B. It appears that these are not the smallest chromosomes after the central one, but are among the intermediate ones. In the polar views, the large double chromosomes appear almost like crosses; in many cases it is impossible to tell whether the division line between the two curved bars or U's is in line with the diameter of the circle or at right angles to it. In fact the chromosome is sometimes turned so that the line of separation lies midway between these two positions (fig. 87). In the anaphase, a peculiar shape is often assumed by X (fig. 89), but after the two sister elements are separated, each shows again the appearance of being composed of a larger and smaller parts (figs. 90 and 91). In these figures are also shown the sister elements of the large compound chromosome (in figure 91, both large ones), each consisting of two curved bars or open U's, bent away from each other. In figure 92, A, B, C, are given serial sections of a spindle in anaphase, showing all the 14 chromosomes in each sister group.

The appearance of X^2 in the early prophase of the first division is even more remarkable than in later stages. Its main central mass is split longitudinally, and at each end of each bar are small knobs attached to the main mass by thin threads. The

[2] Professor Wilson has called my attention to his description of the X chromosome in Lygaeus bicrucis ('12), where in the growth period it consists of several, usually three, paired segments, somewhat resembling the X chromosome of N. indica.

number of these smaller bodies seems to be quite constant, three pairs at one end and two at the other (fig. 93). In the very late prophase, after the chromosome is on the spindle, it is still loosely. organized, and its several characteristic components may be seen (fig. 94). In full metaphase the chromosome becomes more compact, and, as I have described, usually consists of a central mass with a small component at each side. Occasionally one finds the chromosome in metaphase with two small elements on one side and one on the other (fig. 95). In early anaphase, the smaller components appear as knobs or strands projecting at an angle from the main mass (figs. 89, 92 B and 96).

In the second division, the peculiar X Y pair take up the usual position in the center of the spindle surrounded by a ring of 12 other chromosomes (figs. 97, 98 A, B, C and 99 A, B). In the group are the double chromosomes, lying on the spindle with the split, which was in evidence in the first division, on the equatorial plane, marking the line of separation of its components. There is also present in this division an asymmetric quadripartite chromosome, similar to the d-chromosome described by Wilson ('11) in Nezara (figs. 97, 98 C and 99 B). The appearance of the X chromosome in the second division is somewhat variable, owing probably to the way it has been cut in the section; it most often appears as a central mass with small masses hanging down from each end, Y being usually attached by a fiber to one of these ends (figs. 98 B, 99 A and 100 B). The fact that its shape is not dependent on the presence of Y is shown by its reversed position in some cases (fig. 100 E). The connection between the different parts of X is in many cases not evident, but this may be due to the thinness of the thread or to its being just without the plane of section (fig. 97 and 100 C, D). The characteristic ring figure with X Y in the center is seen in polar views (figs. 101 and 102). Among the chromosomes in the peripheral ring are the two large ones which appeared double in side views; their double nature usually does not show now or is only indicated by a notch in the side. Owing to the peculiar shape of X, detached pieces of it often appear in polar view, and since these are about the same size as Y, it is impossible to tell which these

pieces are; in figure 102, the three parts in the center are parts of the X Y pair. Figure 103 shows the different appearances of X Y in polar view. In anaphase, X is usually slow in going to the pole, and is found stretched between the two groups of chromosomes (fig. 104). More frequently X appears very much as it did in the first anaphase, as a central mass with smaller pieces attached, often by the merest thread, or with no visible connection (figs. 105 and 106); it will be observed that these end pieces may extend either toward or away from Y. Sister groups from an anaphase in polar view show again the number and size relations of the chromosomes, and are practically identical, except for the central component, X or Y (fig. 107). The chromatin content of the two classes of spermatozoa differs more in this species than in any of the others, owing to the unusually large volume of X. The structure of X in telophase is very similar to its previous structure, and it is frequently difficult to discover any connection between its components (figs. 108 and 109).

In the spermatogonial (figs. 110 to 113) and the oogonial (figs. 114 and 115) groups, there are 26 chromosomes. Especially characteristic are the long winding chromosomes, single in the male and paired in the female groups; this is of course the X chromosome. Although X shows such a marked tendency to break up into smaller parts in the spermatocyte divisions, I have found little indication of this in the diploid groups. I have examined many groups, and have always found X represented by a single chromosome of almost equal width throughout its length, with sometimes a slight tendency toward notching at one or both ends. In both male and female groups the two large double chromosomes of the spermatocyte divisions are represented by the four next largest chromosomes, and the two small ones by four little ones.

b. The X chromosome of N. indica

The characteristic and unusual appearance of the X chromosome as described in the preceding section leads one to suspect that it is really compound, and is in the process of splitting off or uniting with smaller chromosomes. Whether these smaller

pieces ever become entirely separated from the chromosome proper in N. indica, it is difficult to tell. They are often attached only by a thin fiber, no thicker than the fiber connecting the chromosome with its mate Y, and they often appear to be entirely separate. If these pieces are separate in N. indica, or if they eventually become separate in this species or in one derived from it, X may be considered as a complex of chromosomes rather than as a single chromosome. This complex is however, represented in the diploid groups by a single chromosome. N. indica is, therefore the first link in the chain, from a simple X which occurs in many forms either with or without a mate Y, through those cases where X is composed of two elements sometimes combined, sometimes separate (Phylloxera, Agalena, Syromastes), to the cases where X consists of two or more elements always separate, but acting as a unit in the maturation divisions, e.g., Thyanta calceata (Wilson '11), Galgulus and some of the reduvioids (Payne '09), Ascaris lumbricoides (Edwards '10) and finally Ascaris incurva, where X consists of eight elements (Goodrich '14). Perhaps the case next in series after N. indica is Phylloxera caryaecaulis, where the two parts of X are very unequal, and are indistinguishably fused during part of their history. We have additional evidence from N. indica for Wilson's ('11) assumption that X is essentially compound in nature with a tendency for the parts to segregate as separate chromosomes, and strong morphological evidence for sex-linked characters.

It is also possible that the small pieces of the X chromosome represent the starting point in the appearance of supernumerary chromosomes, such as are found in Banasa calva (Wilson '07), Metapodius (Wilson '07, '09), the Diabroticas (Stevens '08), and Ceuthophilus (Stevens '12). These chromosomes divide in only one of the maturation divisions, as one should expect if they have originated from X, and they frequently accompany the XY pair in some forms. Other hypotheses have been suggested for the origin of the supernumeraries; by the passage of both X and Y to the same pole (Wilson '09), by the replacement of Y by a second m-chromosome (Wilson '10) and by an abnormal division of the X chromosome in one of the maturation divisions (Stevens '12).

Reference should be made to the XY chromosome as described by Stevens ('07) for Drosophila, where there is a third element which looks as though it were separating from the X Y pair in the maturation divisions, although no trace of this was to be found in the diploid groups. Metz ('14), however, who has studied the chromosomes of Drosophila carefully, says that he finds no indication of such a separate element in the X Y pair.

VII. COMPARISON OF THE CHROMOSOMES IN THE SIX SPECIES

The chromosome groups of the different species of Notonecta are in general very similar in number, arrangement and size relations. An effort has been made to account for the slight differences which do occur in the different species.

a. Autosomes

The typical arrangement of the chromosomes in the first maturation division of the six species is a ring of chromosomes of varying sizes, surrounding one or two small chromosomes in the center, and in the second division a ring surrounding the XY pair. The only departures from this grouping are in the case of N. shooterii where one small one is usually a little within the peripheral ring in the first division; and in the case of N. indica, in the first division, where two intermediate ones are somewhat within the ring. The arrangement in N. shooterii may be explained on the assumption that the second small chromosome, which is in the center in N. undulata and N. indica, is in the process of shifting its position to the outside ring, possibly a stage preparatory to fusion with the largest or some other chromosome, or the reverse, a stage in separation. If this be true, we have represented in N. shooterii another step in the change in number of chromosomes in the different species, comparable with that represented in N. insulata, as pointed out in my former paper. The divergence from the typical arrangement in N. indica may be explained on the ground that this species is an offshoot from N. undulata, and its chromosome grouping correspondingly a

modification of that of N. undulata, indicated by the displacement of two of the chromosomes from the outer ring.

With regard to number, the six species may be grouped into two classes, one class having a diploid number of 26, first spermatocyte number of 14, second spermatocyte number 13, and the other class with a diploid number of 24, first spermatocyte number 13, second spermatocyte number 12. To the first class belong N. undulata, N. shooterii and N. indica; to the second class belong N. irrorata and N. glauca. N. insulata belongs rather to the latter group, though it is somewhat intermediate, since many of the first division cells show 14 chromosomes. The difference in number in the two classes can probably be attributed, as previously suggested, to the presence or absence of a particular small chromosome which, when present, lies in the center of the group in the first division, as in the case of N. undulata and N. indica, or just within the peripheral ring as in N. shooterii. In N. insulata its presence or absence can be directly attributed to its separation from or its fusion with the largest chromosome, and I have attributed its absence in N. irrorata to its permanent fusion with the largest chromosome. This explanation will likewise apply to N. glauca, and I believe that this is the correct explanation. There is, however, another possibility which may apply to both of these cases or to one of them. This is that the second small chromosome in present in the peripheral ring, and one of the other peripheral chromosomes represents two chromosomes of the 14-group. In this event, N. shooterii represents the step in transition, the small chromosome being intermediate in position between the central and peripheral positions. There is a small chromosome in the peripheral ring in both N. glauca and N. irrorata, but there is nothing in its position or behavior to show any relationship with the one in the center in the 14-groups, as is the case in N. shooterii. Moreover, there is a small chromosome in the periphery in N. insulata even when the two small ones are present in the center. It seems therefore more plausible to asssume that in N. irrorata and in N. glauca, the fusion of the second small chromosome and the largest one is permanent, and that N. insulata presents one in-

termediate step and N. shooterii another. Such a process of
fusion and separation of chromosomes is analogous with that de-
scribed in the case of the sex chromosomes referred to under N.
indica, and in the case of the sex chromosomes with other chro-
mosomes in the phasmids (Sinéty '01), a few other Orthoptera
(McClung '05), one of the mosquitoes (Stevens '11) and Ascaris
megalocephala (Boring '09, Boveri '09, Edwards '10). In the
different species of Drosophila, Metz ('14) has attributed the
difference in number to a separation of certain V-shaped chro-
mosomes into single bars.

In all the species of Notonecta described, there are two chro-
mosomes larger than any of the others, particularly striking as
four large ones in the diploid groups. Together with X, which
is usually the fifth largest, we find five large chromosomes in the
male and six large ones in the female diploid groups. N. irro-
rata forms an exception in having seven large chromosomes in
the spermatogonial groups. When these chromosomes attain
an especially large size, they appear in the spermatocyte divi-
sions as large compound chromosomes. These are made up of
four curved bars or U's in the first division, so arranged as to
form a cross, though this structure is not always evident owing
to an overlapping of parts. Two of these bars go to each sister
cell, and in the second division, their line of separation lies along
the equatorial plane so that one goes to each spermatid. These
chromosomes are really typical tetrads whose four parts remain
separated until finally distributed in the second division. I have
called them double or compound chromosomes because their
component parts are distinguishable, giving them a composite
appearance, and also because the final element often looks as
though it were composed of two equal parts, owing to a notch
in the center. Whether this notch is merely a remnant of the
original bend in the curved bar, or whether it indicates that the
chromosome is in the process of splitting into two equal parts
may possibly be determined by work on other species. Since
there must be a limit to the size of a chromosome, and since
these large ones, especially the largest one in N. glauca, have
become quite massive, it seems plausible to suppose that this

chromosome is separating into two parts. If so, then the large chromosome of N. insulata is of particular interest since it is composed of a larger and a smaller part and the notch indicates another line of separation. If the unequal parts are in the process of fusion, an equal division of the chromosome means a redistribution of particles in the resulting chromosomes; if the unequal parts are in the process of splitting, then the chromosome is splitting at two points, one split being realized in the first division, the other being only indicated. Various stages in the formation of these large compound chromosomes are to be found in the different species. In the case of the largest chromosome of N. glauca and of the two largest ones in N. indica, the compound nature is evident in the first division. In the case of the largest chromosome of N. insulata and the second large chromosome of N. glauca, the compound nature is not noticeable in the metaphase of the first division except in polar view, but is evident in the second division. In the second largest chromosome of N. insulata and the largest chromosome of N. irrorata, a split is noticeable in some cases in polar view of the first division, but the chromosome does not appear double in the second. In the largest chromosomes of N. shooterii and N. undulata there is no evidence of a compound structure. In general, it may be said that the larger the chromosome is, the greater the tendency is for its component parts to separate.

b. Sex chromosomes

In comparing the chromosome groups of the six species of Notonecta, one of the most striking features is in regard to the XY pair. This pair is almost equal, occasionally quite so in N. shooterii, more unequal in N. glauca, still more so in N. insulata and N. irrorata, very unequal in N. undulata and extremely so in N. indica. This difference in the size relations of X and Y in the different species is caused apparently both by X and Y. In comparison with the other chromosomes, X is the fourth largest chromosome in N. irrorata, the third in N. glauca. N. insulata (probably—the lack of diploid groups prevents an

absolute determination), N. shooterii and N. undulata, and is
the largest in N. indica.   Y is the third smallest in N. undulata,
about the fifth in N. irrorata, N. indica, N. insulata and N.
glauca, and about the eighth in N. shooterii. A similar pro-
gressive difference in the relative size of these chromosomes
has been shown to occur in the different species of Euschistus
(Wilson '06).

VIII. CORRELATION OF SOMATIC CHARACTERS AND CHROMOSOMES

The diagnostic specific characters of Notonecta used by ento-
mologists are the body size, distance between the eyes, width of
the pronotum and size of the scutellum. The coloration and
markings are to some extent characteristic of the different spe-
cies, but there is a great variation in color in certain species from
a pure white to an almost black, so that this character is regarded
as of little value in the determination of species. In the accom-
panying table, I have listed the size relations of the different
parts in the six species, most of the figures being taken from
Bueno ('05). An outline of the number and relations of the chro-
mosomes is also given in the table.

In general a definite number and arrangement of the chromo-
somes is characteristic of each species, and is therefore probably
correlated with the characters of that species. A reference to
the table will show, however, that the species cannot be lined
up with regard to their chromatin content and with regard to
each of the somatic characters with any great agreement. It
would seem that the distance between the eyes, which is perhaps
the most reliable characteristic for the entomologist, is not cor-
related with the most striking chromosome difference, that of
number. There are two characters, however, in which the 14-
chromosome species differ from the 13-group. The species with
14 chromosomes are the small forms—N. undulata, N. indica
and N. shooterii—whereas the species with 13 chromosomes are
the large forms, N. insulata, N. glauca and N. irrorata. Sec-
ondly, there is a physiological difference between the 14-species
and the 13; the former have a rapid evolution of the germ cells,

TABLE 1

SPECIES	EYES VERTEX: SYNTHLIPSIS	PRONOTUM WIDTH: LENGTH	SCUTELLUM WIDTH: LENGTH	LENGTH OF INSECT	COLOR	NO. OF CHROMOSOMES 1st division	NO. OF CHROMOSOMES 2d division	SIZE RELATION AND ARRANGEMENT OF CHROMOSOMES IN 1st DIVISION	SIZE OF X AND Y COMPARED WITH OTHER CHROMOSOMES	DIPLOID NO. OF CHROMOSOMES	SIZE RELATION OF CHROMOSOMES IN DIPLOID GROUPS
N. undulata...	$2\frac{1}{2}$	2	$1\frac{1}{5}$	10-13 mm.	White or black-and-white	14	13	$a + a + M + M' + X + Y + 8A$ Small one in periphery $= Y$	X = 3d largest Y = 3d small-est X and Y quite unequal	26	5 large 5 small } ♂ 16 medium
N. indica...	$1\frac{1}{2}$-2	$1\frac{3}{4}$	$1\frac{1}{2}$	9.4-11 mm.	Black-and-white	14	13	$a + a + M + M' + X + Y + 8A$	X = largest (compound) Y = about 5th smallest X and Y very unequal	26	5 large 4 small 17 medium } ♂ 6 large 4 small 16 medium } ♀
N. shooterii...	$1\frac{1}{3}$	$1\frac{2}{3}$	$1\frac{1}{3}$	8-13 mm.	White or black-and-white or brown-and-white	14	13	$a + a + M + M' + X + Y + 8A$	X = 3d largest Y = 6-11th smallest X and Y almost equal	26	5 large 4 small 17 medium } ♂ 6 large 4 small 16 medium } ♀
N. insulata...	$1+$	2	$1\frac{1}{5}$	12.6-15.5 mm.	Brown-and-black or al-most all brown	14, 13	12	$a + a + M + M' + X + Y + 8A$ or $a + Ma + M' + X + Y + 8A$ Small one in periphery	X = 3d-5th largest Y = 5th or 6th smallest X and Y fairly unequal	26	5 large 4 small 17 medium } ♂ 6 large 4 small 16 medium } ♀

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N. irrorata...	3	2	$1\frac{1}{4}$	12.1–14.4 mm.	Black with brown mottling	13	12	a + Ma(assumed) + 2M' + X + Y + 7A 2 small ones in periphery	X = 4th largest Y = 4th or 5th smallest X and Y fairly unequal	24	7 large 6 small 11 medium } ♂
N. glauca....	2–$2\frac{1}{4}$	2	$1\frac{1}{3}$	13.4–17.2 mm.	Pale yellow or yellow-and black or dark brown	13	12	a + ma(assumed) + M' + X + Y + 8A Small one in periphery	X = 3d largest Y = 4th–6th smallest X and Y almost equal	24	6 large 4 small 14 medium } ♀

EXPLANATION. Vertex = distance between eyes at front. Synthlipsis = distance between eyes at base of head. a = small autosome. a = small autosome in center. M = largest chromosome or macrochromosome. Ma = macrochromosome associated with small autosome. m = macrochromosome double. M' = next largest chromosome. A = intermediate autosome.

so that all stages in the spermatogenesis are to be found throughout the summer and fall; in the 13-species, on the other hand, the evolution of stages is very slow, so that at one time the testis is filled with cells in the growth stages, then with dividing cells, and in the late summer only spermatozoa are to be found.   Probably as a result of this, the cells of the former species are smaller than the latter.   Whether these two characters are really correlated with the chromosome number, or whether it is a mere coincidence can probably be determined by work on other species.

Just what the status of each species is in relation to the others cannot be told at present.   I think there is little doubt that N. indica is a species derived from N. undulata, as both the somatic characters and the chromosomes indicate this.   N. shooterii is particularly puzzling because it seems primitive with regard to the XY pair and yet it seems to represent a transition stage with regard to the autosomes.

IX. CONSTANCY OF THE CHROMOSOMES

Notonecta adds to the ever increasing mass of evidence that the chromosomes are definite bodies with individual peculiarities and characteristics.   Each species is characterized by some special behavior or structure or position of certain of the chromosomes, and this characteristic is constant for all the individuals and all the cells of that species with few exceptions.   I would especially emphasize the constancy in the arrangement of the chromosomes.   There is always some particular grouping of the chromosomes, different in the two divisions, but characteristic of some particular species.

X. SUMMARY

1) An XY pair of chromosomes is present in all six species, the components dividing separately in the first division and going to opposite poles in the second.   The components frequently lie side by side in the second metaphase.

2) The X and Y chromosomes are almost equal in most cells of certain individuals of N. shooterii.   They are more nearly

equal in certain individuals of N. glauca than in others. In the other species, these chromosomes are more unequal, and are extremely so in N. indica.

3) The X chromosome of N. indica is a compound chromosome composed of larger and smaller parts.

4) In N. undulata, indica and shooterii, there are 14 chromosomes in the first division and 13 in the second and 26 in the diploid groups. In N. irrorata and glauca, there are 13 chromosomes in the first division and 12 in the second and 24 in the diploid groups. In N. insulata, there are 14 or 13 in the first and 12 in the second.

5) In all the species except N. indica, the chromosomes in the first division are arranged in a ring with the small ones in or near the center. In N. indica, two of the intermediate chromosomes are somewhat within the ring. In all the species in the second division, the chromosomes are arranged in a ring with XY in the center.

6) In N. undulata, indica and in the 14–groups of insulata, there are two small ones in the center of the ring in the first division. In N. irrorata and glauca and in the 13-groups of insulata, there is only one small one in the center. In N. shooterii, there is one small one in the center and another between the center and the periphery. In the 13-groups of N. insulata, the second small one is often found attached to the macrochromosome.

7) Large double chromosomes are characteristic of N. insulata, glauca and indica.

8) The differences in somatic characters in the different species cannot be definitely correlated with the differences in chromosome number or arrangement. However, the 14-chromosome species are the smaller species, while the 13-chromosome species are the larger ones.

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N. undulata, N. irrorata, N. insulata. $\times$ 2000

1 N. undulata, metaphase of the first division, polar view, showing 14 chromosomes, two small ones in the center.

2 N. undulata, metaphase of the first division, side view, showing linear arrangement of the central pairs.

3 N. undulata, metaphase of the second division, polar view, showing 13 chromosomes, XY in the center.

4 N. undulata, metaphase of the second division, side view.

5 A, B N. undulata, sister anaphase groups of the second division, from the same spindle.

6 N. undulata, spermatogonial groups showing 26 chromosomes.

7 N. irrorata, metaphase of the first division, polar view, showing 13 chromosomes, one small one in the center.

8 N. irrorata, metaphase of the first division, side view.

9 N. irrorata, metaphase of the second division, polar view, showing 12 chromosomes. Both X and Y appear in the center.

10 N. irrorata, metaphase of the second division, side view.

11 A, B N. irrorata, sister anaphase groups of the second division from the same spindle.

12 N. irrorata, spermatogonial group, showing 24 chromosomes.

13 N. insulata, metaphase of the first division, showing 14 chromosomes, two small ones in the center.

14 N. insulata, metaphase of the first division, showing 13 chromosomes, one small one in the center.

15 N. insulata, metaphase of the first division, side view, with two small ones in the center.

16 N. insulata, metaphase of the first division, side view, with one small one in the center and the large chromosome with a small one attached.

17 N. insulata, metaphase of the second division, showing 12 chromosomes.

18 N. insulata, metaphase of the second division, side view.

19 A, B N. insulata, sister anaphase groups of the second division, from the same spindle.
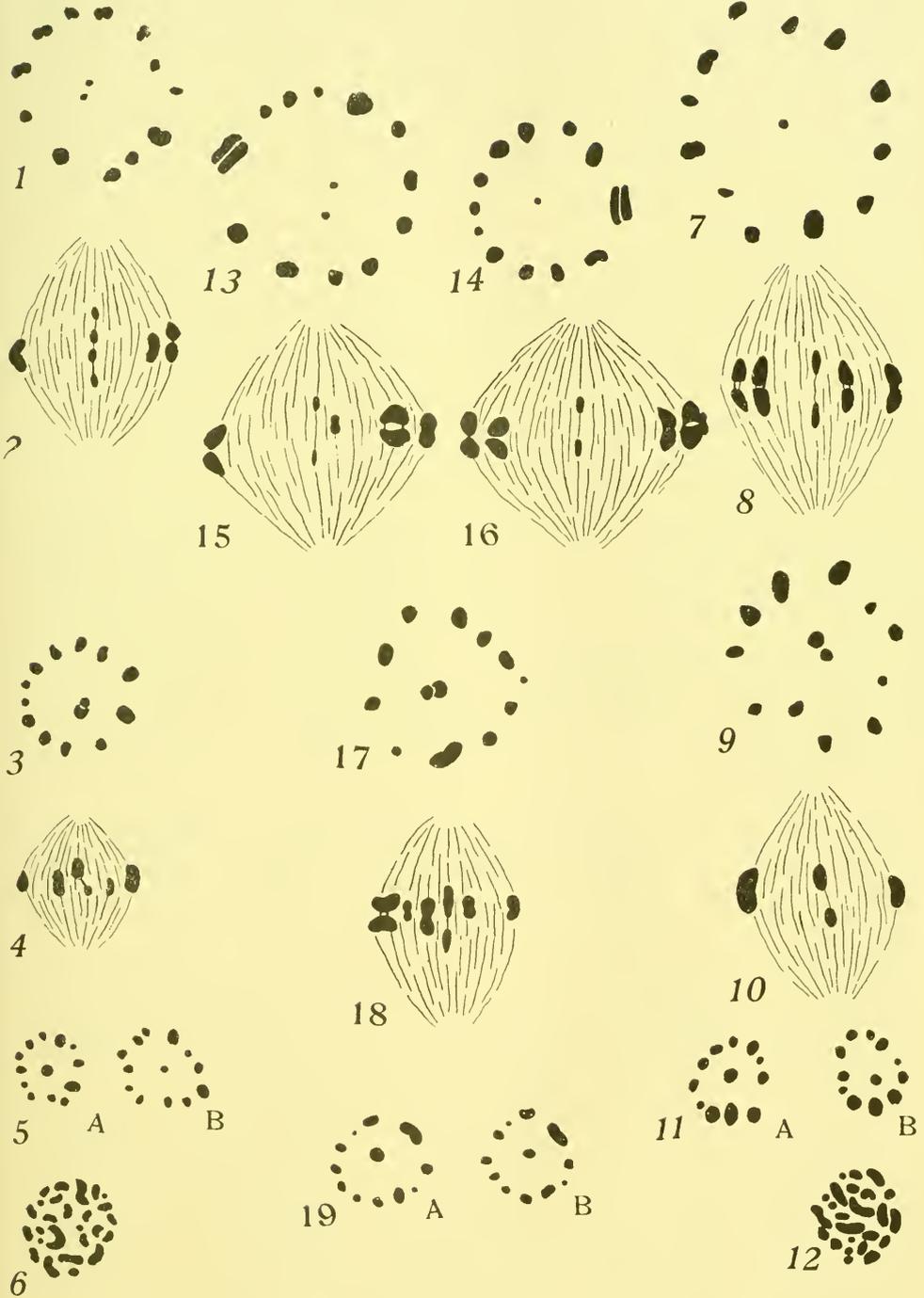
N. glauca, first division. $\times$ 2000

20 to 22   Polar views of the metaphase, showing 13 chromosomes.

23   Side view of initial anaphase.

24 A, B, C   Serial sections of entire spindle in side view, showing 13 chromosomes.

25 B   Side view showing components of central dyad on different fibers. A. Other chromosomes from the same spindle.

26   Large compound chromosome in side view at the side of the spindle. The dark and light portions represent similar dark and light areas on the chromosomes.

27   Large compound chromosome in side view on the top of the spindle.

28   Large compound chromosome in polar view.

29 and 30   Early anaphase, side view, showing division of the large compound chromosome.

31 and 32   Later anaphase, side view, showing both large chromosomes.

33   Polar view of anaphase.

34   Late anaphase, showing chromatic substance from the large chromosome streaming down over the spindle fibers.

ETHEL NICHOLSON BROWNE
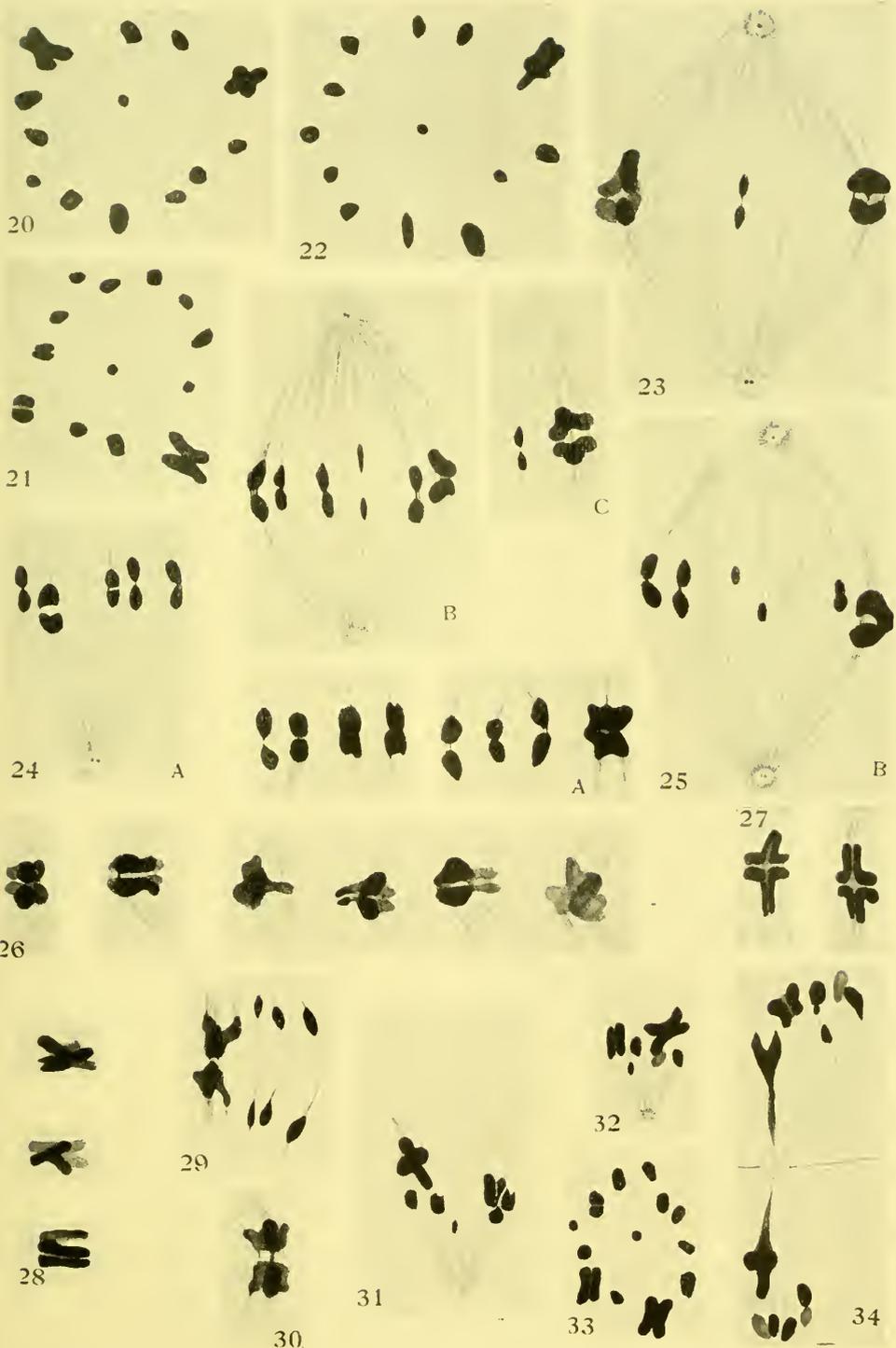
N. glauca, second division. $\times$ 2000

35   Telophase of the first division, prophase of the second, showing both large chromosomes.

36   Metaphase, side view, showing large compound chromosome and XY.

37 A, B, C   Serial sections of entire spindle in side view, showing complete set of 12 chromosomes.

38 A, B, C   Same, only X and Y are on different fibers.

39 and 40   Polar view, showing 12 chromosomes.   Both X and Y appear in the center.

41   Side view of a metaphase spindle, showing two large and two small chromosomes.

42   XY chromosomes from one individual.

43   XY chromosomes from another individual, showing greater inequality of the two chromosomes.

44   Early anaphase showing large chromosomes and XY.

45   Late anaphase, showing two large chromosomes near upper pole and largest one near lower pole.

46   Late anaphase, showing largest chromosome near pole.

47 A, B   Sister anaphase groups from same spindle, central chromosomes (X and Y) considerably unequal.

48 A, B   Same.   X and Y more nearly equal.

49   Spermatogonial group, showing large chromosomes and small ones.

50   Oogonial group showing 24 chromosomes.

35

37

A   B   C

38   A   B   C

36   39   40   41

42

43   44   45

46
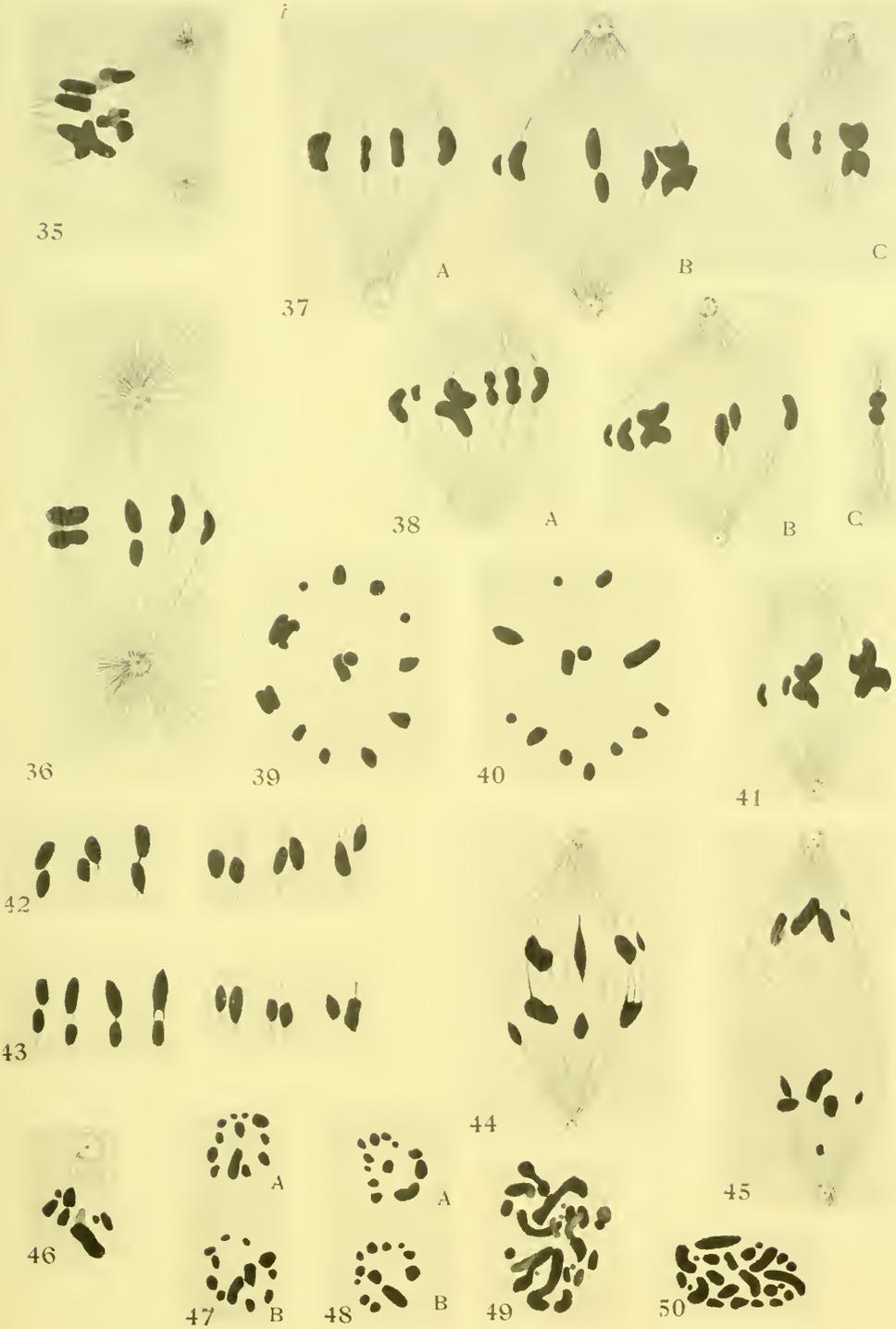
47   B   48   B   49   50

51   Polar view, first metaphase, showing 14 chromosomes, one small central one and one just within the peripheral ring.

52   Same, second small one further inside the ring.

53   Side view of metaphase, two small ones at the same focus.

54 A, B, C   Serial sections of entire spindle in side view, showing all the chromosomes.   In B, the second small chromosome lies at a lower level than the large ones.

55   Oblique section, showing the second small chromosome within the peripheral ring.

56   Side view of metaphase, showing components of central chromosome on different fibers, and the second small chromosome nearer the center than the large ones.

57 A. B   Serial sections of entire spindle in side view of late anaphase, showing all the chromosomes in each daughter group.   In B, the two small chromosomes are shown in proximity.

58 to 60   Side views of late anaphase groups, showing proximity of the two small chromosomes.

61   Polar view of late anaphase, showing the two small chromosomes in the center.

51

52

53

54   A

B

C

55

58

59

56

57   A

B

60

61
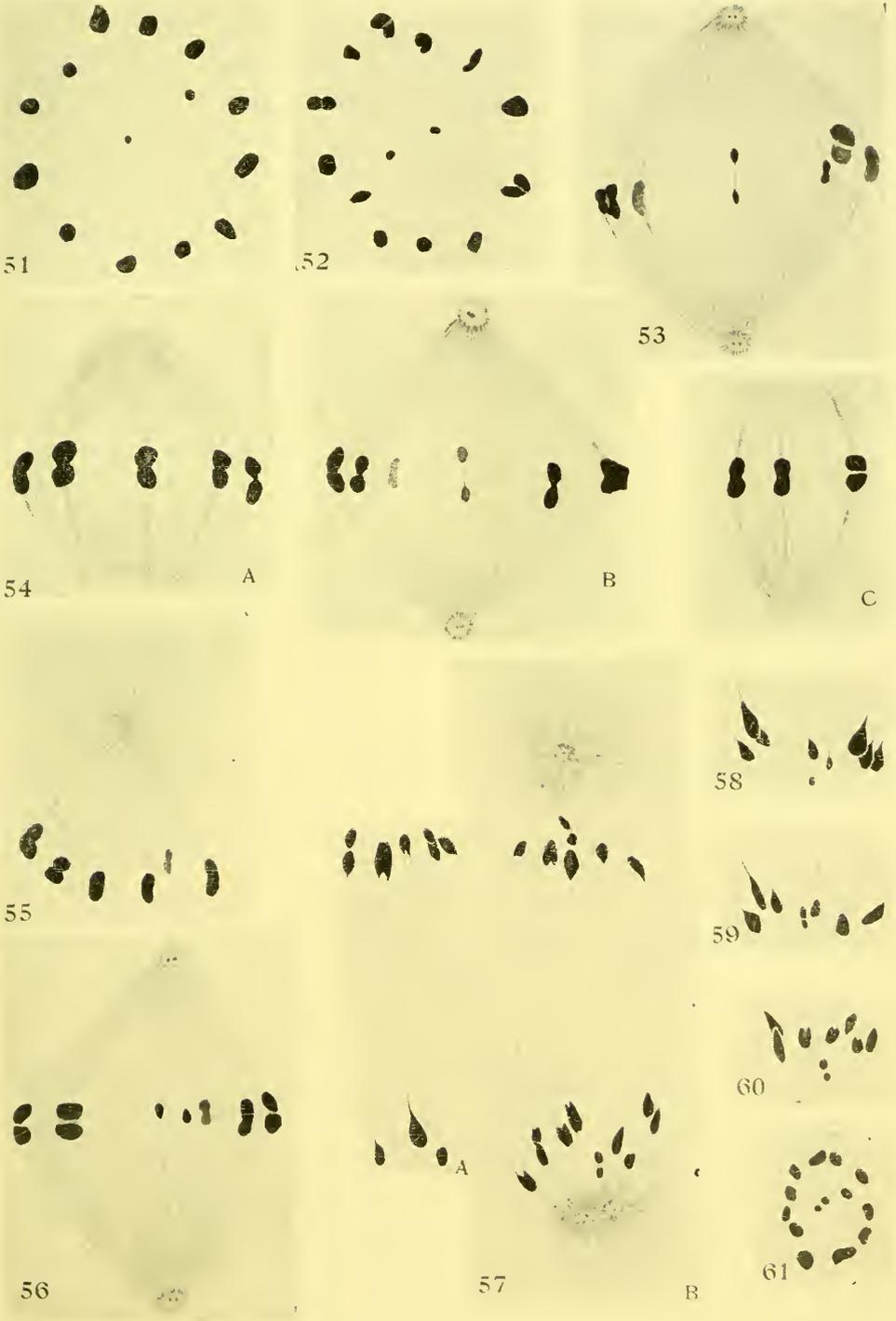
62 A, B, C   Serial sections of entire spindle in side view, showing all 13 chromosomes.

63 and 64   Polar view of metaphase, showing 13 chromosomes.

65 A, B   Serial sections of entire spindle, XY lying side by side.

66   Side view of anaphase.

67 A, B   Sister groups of early anaphase.

68   The XY pairs from one individual, showing marked inequality.

69 and 70   The XY pairs from two other individuals, showing less inequality.

71 to 74   The XY pairs from four other individuals, showing still less inequality and in some cases an apparent equality.

75 A, B   Sister anaphase groups from the same spindle, X and Y markedly unequal.

76 A, B   Same, X and Y more nearly equal.   (A small chromosome is missing in A at the upper left hand corresponding with the one in B.)

77 A, B   Same, X and Y practically equal, making the two groups almost identical.

78 and 79   Spermatogonial groups, showing 26 chromosomes including five large ones.

80   Oogonial group, showing 26 chromosomes including six large ones.
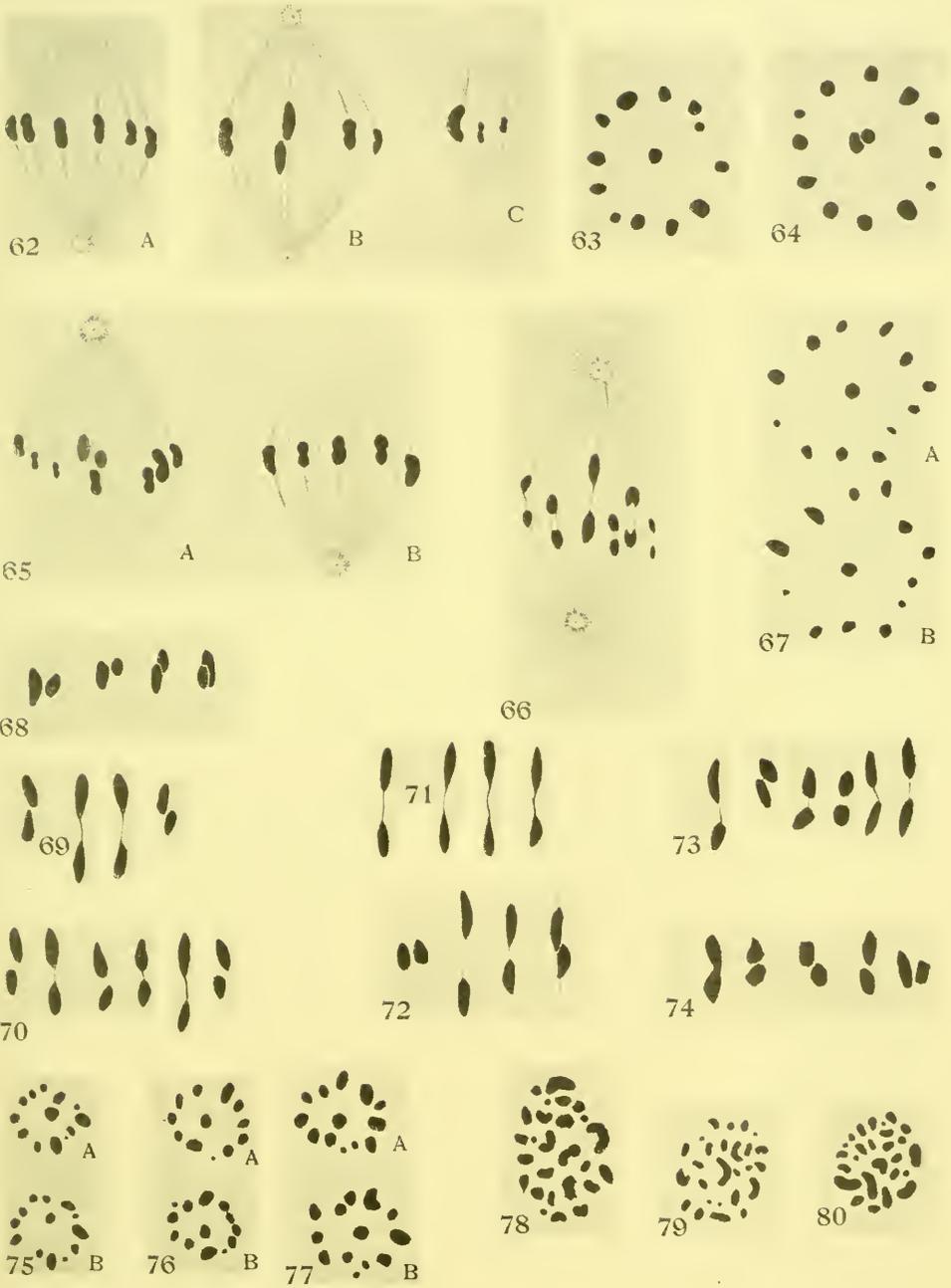
N. indica, first division. $\times$ 2000

81   Side view of metaphase, showing two small chromosomes in the center; the chromosome in light grey is at the same focus as the central ones, and therefore within the peripheral ring.

82   Same, only the two central pairs are linearly arranged. The X chromosome is shown at the left and a large double at the right. The light and dark portions represent similar light and dark areas seen on the chromosomes. The chromosome in light grey is within the peripheral ring.

83 and 84   Side views showing X and one double chromosome.

85 A, B, C   Serial sections of entire spindle, showing all the 14 chromosomes; two large doubles in A, two small central ones and another chromosome in light grey at the same focus in B, and X in C.

86 to 88   Polar views of the metaphase, showing 14 chromosomes.

89   Side view of anaphase showing division of X.

90 and 91   Late anaphase, showing X and large chromosomes in sister cells; both large ones are shown in 91.

92 A, B, C   Serial sections of entire spindle in anaphase, showing all the chromosomes in both sister cells.

93   X chromosome in early prophase, before the spindle is formed.

94   X chromosome in very late prophase, after the spindle is formed.

95   X chromosome in metaphase.

96   X chromosome in anaphase.

81

82

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84

85

A

B

C

89

86

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90

92

A

B

C

91

93   94   95   96
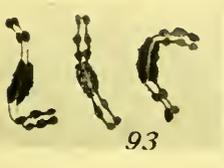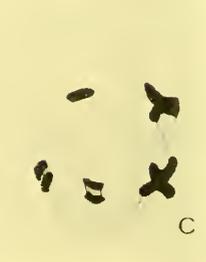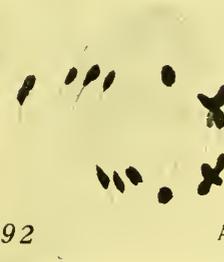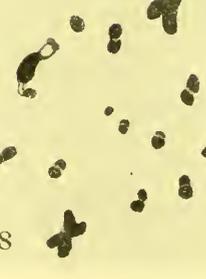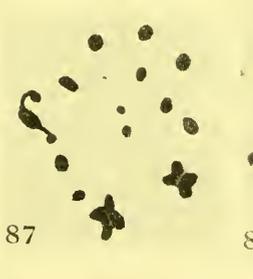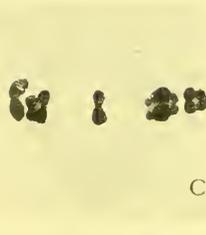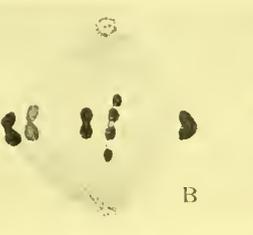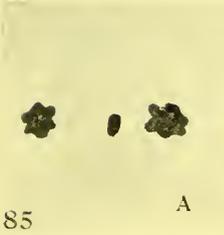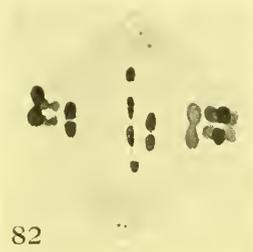
N. indica, second division. × 2000

97 Side view of metaphase, showing X and Y, one large double chromosome and an asymmetric tetrad.

98 A, B, C and 99 A, B Serial sections of two entire spindles, showing all 13 chromosomes.

100 A to E The XY chromosomes in side view of the metaphase.

101 and 102 Polar views of metaphase, showing 13 chromosomes. The three bodies in the center in 102 are parts of XY.

103 The XY chromosomes in polar view of the metaphase.

104 Side view of spindle in anaphase, XY in the center.

105 The XY chromosomes in early anaphase.

106 The XY chromosomes in late anaphase.

107 A, B Sister anaphase groups from same spindle, A containing X and B containing Y, otherwise practically identical.

108 Side view of early telophase, showing X near upper pole.

109 The X chromosome in side view in telophase.

110 to 113 Spermatogonial groups, showing 26 chromosomes including five large ones; X is the largest.

114 and 115 Oogonial groups, showing 26 chromosomes including six large ones; the two largest are the two X chromosomes.

161
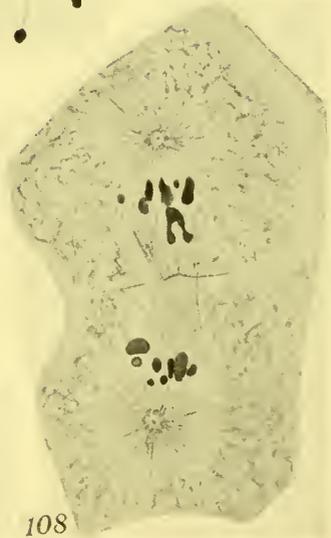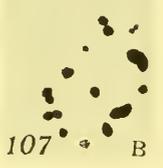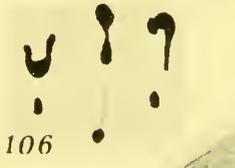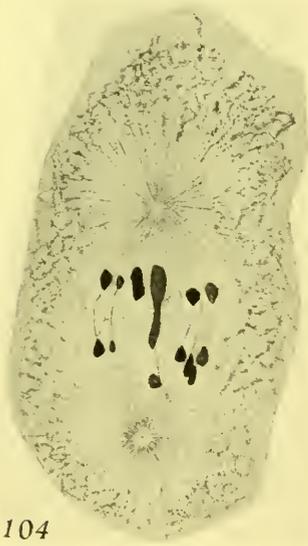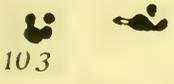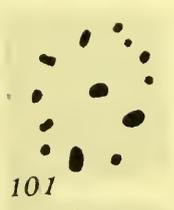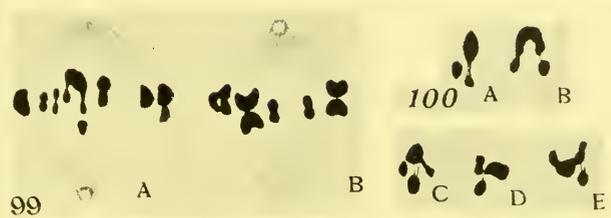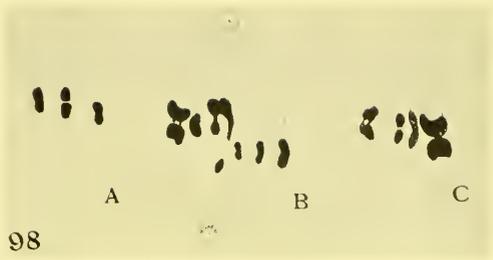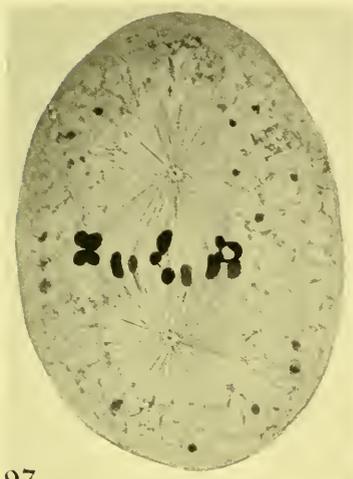
ORGANS OF SPECIAL SENSE OF PRORHYNCHUS APPLANATUS KENNEL

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THREE TEXT-FIGURES AND TWO PLATES

This gregarious rhabdocoele was found in the ponds in the vicinity of the University of Virginia. These animals occurred on plants and other submerged objects, especially at the outlets of the ponds where there was a quantity of moss which was kept damp by the falling of the water. These animals are creatures of a crawling habit and never seem to leave the surface of some object. In the laboratory they crawl along the bottom or sides of the aquaria or some submerged surface and never leave this unless forced to do so by some mechanical means. Later in this paper we will point out what we think to be a correlation between the disposition of the organs of special sense in a creeping form like this as compared with the disposition of such organs in a free swimming form such as Microstoma caudatum Leidy.

Specimens used for this histological study have been fixed in aceto sublimate, at various temperatures, Bouin's fluid, Carnoy's fluid, chrom-formalin mixture, and chrom-aceto-formalin mixture. Without exception, we find that the last named fixative is the best for this purpose. Specimens were fixed in this chrom-aceto-formalin mixture[2] from twenty to thirty minutes, washed in several changes of tap water, and then carried through to paraffin. We find that it is best to handle the specimens separately and not to work on a large number at one time. Sections, 3 to 10 microns thick, were stained in iron haematoxylin with

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[1] The authors are mutually responsible for the observations and deductions recorded in this paper.

[2] 16 parts 1 per cent chromic acid, 8 parts 10 per cent formalin, 1 part glacial acetic. This mixture has to be used as soon as it is mixed.

Bordeau red as a counter stain or with Mallory's connective tissue stain. While we tried several other stains, such as toluidin blue, hydrochloric acid carmine, etc., we think that the haematoxylin and Mallory's connective tissue stain give the best results. This last is especially useful in differentiating the nervous elements.

Prorhynchus applanatus has a greatly dorso-ventrally compressed body which measures from 1.5 mm. to 4 mm. in length. It is somewhat broader and has a more angular margin at its anterior end than at the slightly rounded posterior end. "Der endständige Mund führt in eine enge, schwach längsgefaltete Pharyngealtasche, der wohlausgebildete Pharynx scheint durch die Anordnung seiner Muskulatur, ähnlich wie bei andern Arten, aus 3 Abteilungen zu bestehen" (Von Graff, '13, S. 64). The pharynx leads into a very long enteron which extends from the posterior end of the body to about three-quarters of the posterior end of the pharynx. This enteron gives off many very blunt diverticula. As a rule the larger specimens present more pronounced and longer diverticula than the smaller ones. Von Graff states, in the description quoted from in the above, that nothing is known of the male sexual organs or of the ciliated pits.

In all of the specimens we have studied we had no trouble finding animals with ovaries, but not in a single case did we find a specimen with any male organs. In connection with this it is interesting to note what we think is a special adaptation to this lack of a chitinous penis, be this lack permanent or only temporary in the life history of the animal. It has been long known that in some of the forms of the Turbellaria the penis is as much used in the catching of prey as in copulation. Benham ('01, p. 21) says that, "no doubt the arrangement in Prorhynchus, where the penis opens at the same pore as the pharynx, and is armed with a perforated spine, has come about by the employment of this organ in catching prey." In another species of Prorhynchus where the arrangement of the penis is characteristic, we have seen the animals attack other rhabdocoeles and pierce them with their penes. This brought up the question

as to how a species of Prorhynchus without a penis would defend itself. On placing some Prorhynchus applanatus with some other species of Prorhynchus, which had chitinous penes, the former animals defended themselves with their pharynges. This organ can be thrown out of the mouth to a considerable distance and with enough force to tear other flat worms to pieces. We think that it is a point well to be noted that here we have a species of Prorhynchus in which the pharynx has taken the place of the penis as an organ of defense; or if not this, then the other alternative that the penis has not as yet developed to supplant this function of the pharynx.

Von Graff's second statement that nothing is known of the ciliated pits leads us first to consider the central nervous system with which the pits are intimately associated. In the living specimen at each side of the anterior end of the pharynx, lying close to the epithelium of the proboscis sheath, are two colorless, refractive, plano-convex bodies, the dorsal ganglia (text figs. A and B). In life these ganglia appear to have their axes parallel to that of the pharynx sheath. However, histological preparations show that these ganglia are slightly concavo-convex bodies, with their axes dipping ventrally at their posterior ends. Thus the ganglia obliquely girdle the sides of the pharynx sheath. At their more dorsal, anterior ends the ganglia are connected by a dorsal commissure which arches over the mid-dorsal wall of the pharynx sheath (text figs. B and C). The posterior, ventral extremity of each ganglion is continued as a long, tapering, ventral nerve which passes beneath the enteron (text figs. B and C). This sheath is supplied with two special nerves, though the ganglia appear at places to lie in contact with the pharynx sheath. Each of these nerves leaves the mesial, ventral side of the anterior end of the ganglion and passes to the ventral wall of the pharynx sheath (text figs. B and C, NPh). Just a little anterior and lateral to the origin of each nerve of the pharynx sheath a second nerve arises which, when near the ciliated pit, sends a branch to the anterior end of the body and a second branch to the posterior dorsal wall of the ciliated pit (text fig. C, NCP).

While the ciliated pits are the chief organs of special sense, two other organs of special sense are associated with them. These are the two eyes. One of the unique features of these four organs of special sense is that they are formed by a definite number of cells Only eighteen cells are concerned with the formation of all four organs and this number is always constant.
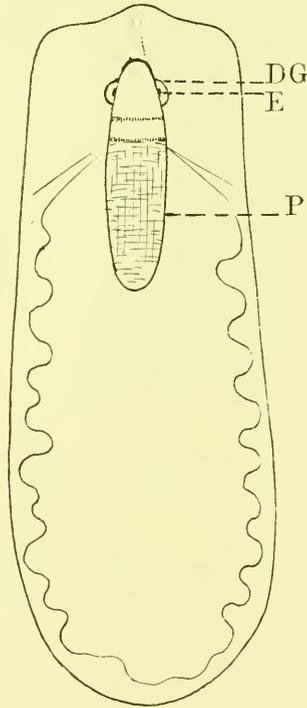
Text fig. A   Drawing of dorsal aspect of Prorhynchus applanatus.   *DG,* dorsal ganglion; *E,* eye; *P,* pharynx.   × 75.

The eyes appear in the living specimen to lie immediately over the dorsal ganglia (text fig. *A*). Each consists of two cells— an accessory or pigmented cell and a retinular or visual cell. The histological features of this accessory or pigmented cell suggest that it is a modified mesenchymal element. Its mesial cytoplasm and nucleus resemble that of a typical mesenchymal

cell (fig. 8, *NAC*). The lateral part of its cytoplasm, however, has assumed a definite cup-like contour, and in its fixed condition, shows concentric lamellæ with which the pigment of the cell is associated (fig. 8, *Pg*). The mouth of this cup is directed
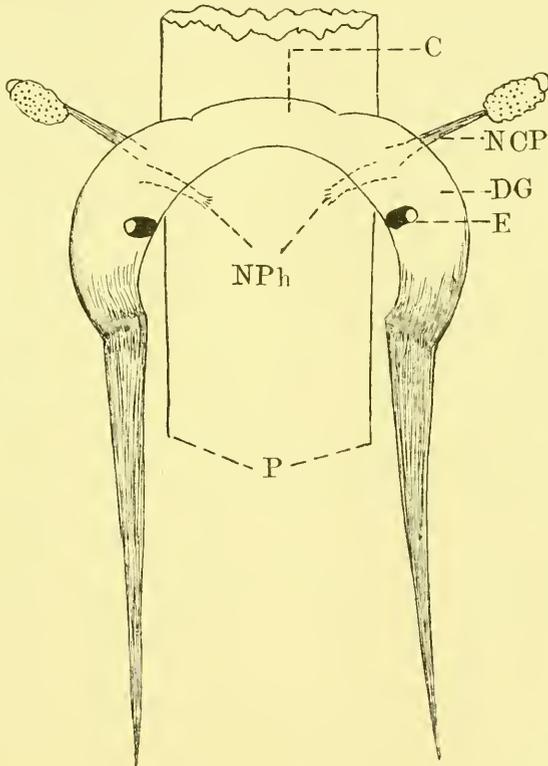
Text fig. B Reconstructed drawing of the central nervous system and organs of special sense; dorsal aspect. *C*, dorsal commissure. *DG*, dorsal ganglion; *E*, eye; *P*, pharynx; *NPh*, pharyngeal sheath nerves; *NCP*, nerve of ciliated pit.

laterally and is closely applied to the convex surface of the retinular cell (fig. 8, *NVC*).

The retinular cell appears to be a modified nerve element. Its nucleus has the characteristic spheroidal contour of that of a typical nerve cell. In the fixed condition, the cytoplasm of

this cell presents three regions—lateral, middle, and mesial.
The lateral region is a finely granular cone which lies closely
applied to the dorsal surface of the ganglion (fig. 8, *C*). The
lateral end of this cone, we infer, is continued as a nerve fiber
into the ganglion. The basal or mesial fourth of this cytoplasmic
region accommodates the nucleus. The middle cytoplasmic
region of the retinular cell is the densest portion of this visual
element (fig. 8, *L*). It is a concavo-convex disc. In a living
specimen under a water immersion lens, a highly refractive body
is seen at the margin of the pigmented cups of the accessory
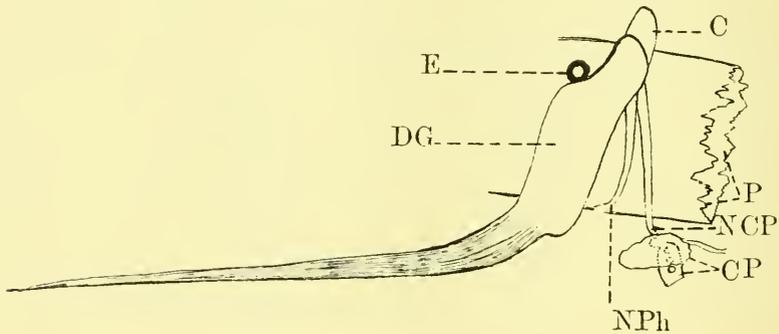
Text fig. C Reconstructed drawing of central nervous system and organs
of special sense. Lateral aspect. *C*, dorsal commissure; *DG*, dorsal ganglion;
E, eye; *P*, pharynx; *NPh*, pharyngeal sheath nerve; *NCP*, ciliated pit nerve;
CP, ciliated pit.

cell; this we regard as this middle curved or lens-shaped part
of the cytoplasm. This region is clearly shown in an axial sec-
tion of the fixed cell (fig. 8, *L*). It is here seen, also to be the
densest portion of the cytoplasm and to have the contour of a
concavo-convex lens. This lens-shaped cytoplasmic region is so
disposed that, if it is functional as a lens, it would cause the rays
of light to converge as they pass through the rhabdome to fall
upon the pigment of the accessory cell. However, we are not
prepared to say that this region does act as such a lens but are
content to look upon it as a supporting structure of the mesial
cytoplasmic region, which is the rhabdome of the visual cell.

Further, this middle or lens-shaped region, which in both the living and fixed specimen appears to be a highly refractive body, is the only essential feature that makes this simply formed eye different from that described by Hesse ('97) for Planaria torva.

Now to return to the cytology of the retinular cell, the mesial region or the rhabdome is a low sugar loaf-shaped body that fills the pigmented cup of the accessory cell. In the fixed condition this region presents a finely granulated appearance (fig. 8, *Rh*).

As stated before, we consider the ciliated pits the chief organs of special sense. These organs open on the ventral side and are disposed laterally and ventrally to the thick nerve commissure which joins the two dorsal ganglia (text fig. *C, CP*). When the animal is crawling upon a surface it seems to make numerous exploratory movements by raising and lowering the anterior sixth of its body. Thus we have a crawling animal with ventrally disposed ciliated pits which makes its exploratory movements by raising and lowering the anterior end of its body. It is well to compare the conduct of this animal with that of Microstoma caudatum Leidy with reference to the position of their respective ciliated pits. Microstoma caudatum is a free-swimming animal and has laterally disposed pits. As we have shown ('12) this animal makes exploratory movements by moving its anterior end from side to side. Likewise we gave experimental evidence to show that these exploratory movements were made in order to test the surrounding medium. Thus we see by the comparison of the two rhabdocoeles that the method in which they test the surrounding water conforms to the position of their ciliated pits.

The remaining fourteen cells of the eighteen concerned in the organs of special sense are involved in the structure of the two ciliated pits. These organs are invaginated regions of the ventral epidermis, directed obliquely, posteriorly and mesially. Of the seven cells that form each pit, six form a syncytium with two rows of three nuclei each. One row is disposed dorsoventrally and forms the lateral wall of the pit, while the other row forms a similar mesial wall (figs. 6 and 7). The general

surface of the syncytial wall bears stout cilia.   If nothing more
could be said of this pit than that it is an organ whose wall is
composed of ciliated cells, no departure would be made from the
prevalent conception of the anatomy of the ciliated pits of flat
worms.   However, all of the wall of the ciliated pit does not
bear cilia, nor is the cytoplasm of uniform density.   The con-
tinuity of the cilia is broken by two ridges which lie more or less
parallel to the axis of the pit.   These ridges do not bear cilia and
their cytoplasm is denser than that of the general wall of the
organ (figs. 3 and 4, *SR*, and fig. 6).   This ridge of cytoplasm
is most prominent and densest over the middle nucleus of each
side (fig. 6, *C*).   Correlated with this the cytoplasm surrounding
this nucleus receives the most extensive nerve supply (fig. 3,
N., and 7, *C'*).   The middle nucleus of each side therefore, and
its adjacent cytoplasm we consider to represent the sensory
region of the ciliated pit; the four remaining elements of the
syncytium being the accessory cells (fig. 6, *B* and *D*).   The
nerve which runs to this sensory region is a branch of the an-
terior trunk given off from the dorsal ganglia.

The sensory ridges or rods, however, do not constitute the
feature that makes these pits most depart from the prevalent
conception of these organs of flat worms.   There is in addition
to the six cells above referred to, a seventh.   This is a gland
cell (figs. 6 and 7, *GC*), which lies over the fundus and communi-
cates with the lumen of the pit by means of a varying number
of ducts, which pass through the wall of the pit on the anterior
angle of its fundus (figs. 2, 5, 6 and 7, *GP*).   The contour of this
unicellular gland is quite irregular and the cell gives off an incon-
stant number of diverticula.   Within the cytoplasm are refrac-
tive granules which take, with Bordeau red, a bright, reddish
yellow tint, marking them in a manner peculiar to this uni-
cellular gland.   Though we here commit ourselves to the con-
cept that this is a unicellular gland, its contour is so irregular
and its limits are so poorly defined that it is quite possible that
other nuclei, which belong to this grandular region, have been
overlooked.   However, there are associated with the general
epithelium of the body, numerous unicellular glands, which,

too, have quite irregular contours and which resemble greatly the gland of the pit.

In addition to this accessory gland there are three non-striated muscle fibers associated with the pit. These arise beneath the dorsal epidermis of the margin of the body of the animal and are inserted laterally upon the syncytial wall of the pit (figs. 2 and 7, *M*). The contraction of these muscles lifts the pit laterally from the substratum. Some of these muscle fibers extend through the gland cell or between its branches.

This pit, then, with its sensory and glandular regions and its definite musculature does not conform to the conception presented by the following statement from von Graff ('09, S. 64) who says of rhabdocoeles that "Die Grübchenflecken sind Haut-stellen, die keine Rabdoiden und Drüsenausführungsgänge besitzen," and likewise Wilhelmi ('08) in his description of the 'Auricularsinnesorgane,' gives no such differentiation. However, in a former paper ('12) the authors have shown that the ciliated pits of Microstoma caudatum Leidy were so differentiated and contained both a sensory and a glandular region. Likewise, there were well defined ducts which emptied the secretion of the large unicellular glands into the fundus of the pit. From that study we drew the conclusion that the structure of the ciliated pit of Microstoma caudatum strengthens the affinity between the Turbellaria and the Nemertini. This conclusion was drawn because the 'cerebral organs' of the Nemertini, in their simplest condition, were, until our study of Microstoma, supposed to differ from the ciliated pits of the Turbellaria only in that they were differentiated into a sensory and a glandular region, while the ciliated pits were not so modified.

The present observations reveal the fact that there is in Prorhynchus applanatus a ciliated pit which is very much more highly specialized, both in its cytology and possession of a definite musculature, than that of Microstoma caudatum. Therefore Prorhynchus applanatus carries us a step further in establishing the affinity between the Turbellaria and Nemertini.

SUMMARY

1) Correlated with the absence of a chitinous penis, which in the other forms of Prorhynchus is used as an organ of defense, the pharynx of Prorhynchus applanatus serves as a defensive structure.

2) The four organs of special sense of Prorhynchus applanatus are always composed of a definite number of cells (viz., eighteen) each of the two eyes is formed by two cells and each of the two ciliated pits by seven cells.

3) The simple eye of Prorhynchus applanatus differs from that of Planaria torva, as described by Hesse, only in that the visual cell of the former presents a highly refractive lens-shaped portion of cytoplasm at the base of the rhabdome.

4) The ciliated pit of Prorhynchus applanatus is sharply differentiated into three regions: sensory, accessory, and glandular.

5) The ciliated pit of the thigmotactic rhabdocoele, Prorhynchus applanatus, differs from that of a free swimming form, like Microstoma caudatum, in having a musculature by which the pit may be lifted from the substratum.

6) The affinity between the Turbellaria and the Nemertini, which was suggested by the structure of the ciliated pits of Microstoma caudatum, is further strengthened by the more highly specialized ciliated pit of Prorhynchus applanatus.

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1 Transverse section involving the fundus of ciliated pit. *GN*, nucleus of gland cell; *GC*, cytoplasm of gland cell; *PW*, syncytial wall of pit; *M*, muscles of pit. In the reconstructions (figs. 6 and 7) this section is shown at *A*, *A'*. × 1500.

2 Section at *B* and *B'* in reconstructed figures 6 and 7. *GC*, cytoplasm of gland cell; *GP*, pores of gland cell; *PW*, syncytial wall of pit, with two nuclei shown in section; *N*, nerve of pit; *M*, muscles of pit. × 1500.

3 Section at *C* and *C'* in reconstructed figures 6 and 7. At this level the sensory cytoplasmic ridge of wall of the pit is structurally different from the cytoplasm of the general wall of the pit. *SR*, sensory ridge. *N*, nerve of pit. × 1500.

4 Section at *D* and *D'* in reconstructed figures 6 and 7. The lumen at this level is greatly narrowed posterior to the sensory ridges, *SR.*, and remains wide and conspicuously ciliated anterior to these ridges. × 1500.

5 Frontal section of a pit. Taken from about the level, *G*, in reconstructed figure 7. *GP*, pores of gland cell opening into lumen of pit. × 1500.

6 Reconstruction, based upon sections figured at 1, 2, 3, and 4. Anterior aspect of pit of right side. *A*, *B*, *C*, *D*, *E*, *F* represent levels of sections; *M*, muscles of pit; *GC*, gland cells; *GP*, pores of gland cell, which lead into lumen of pit; × 2000.
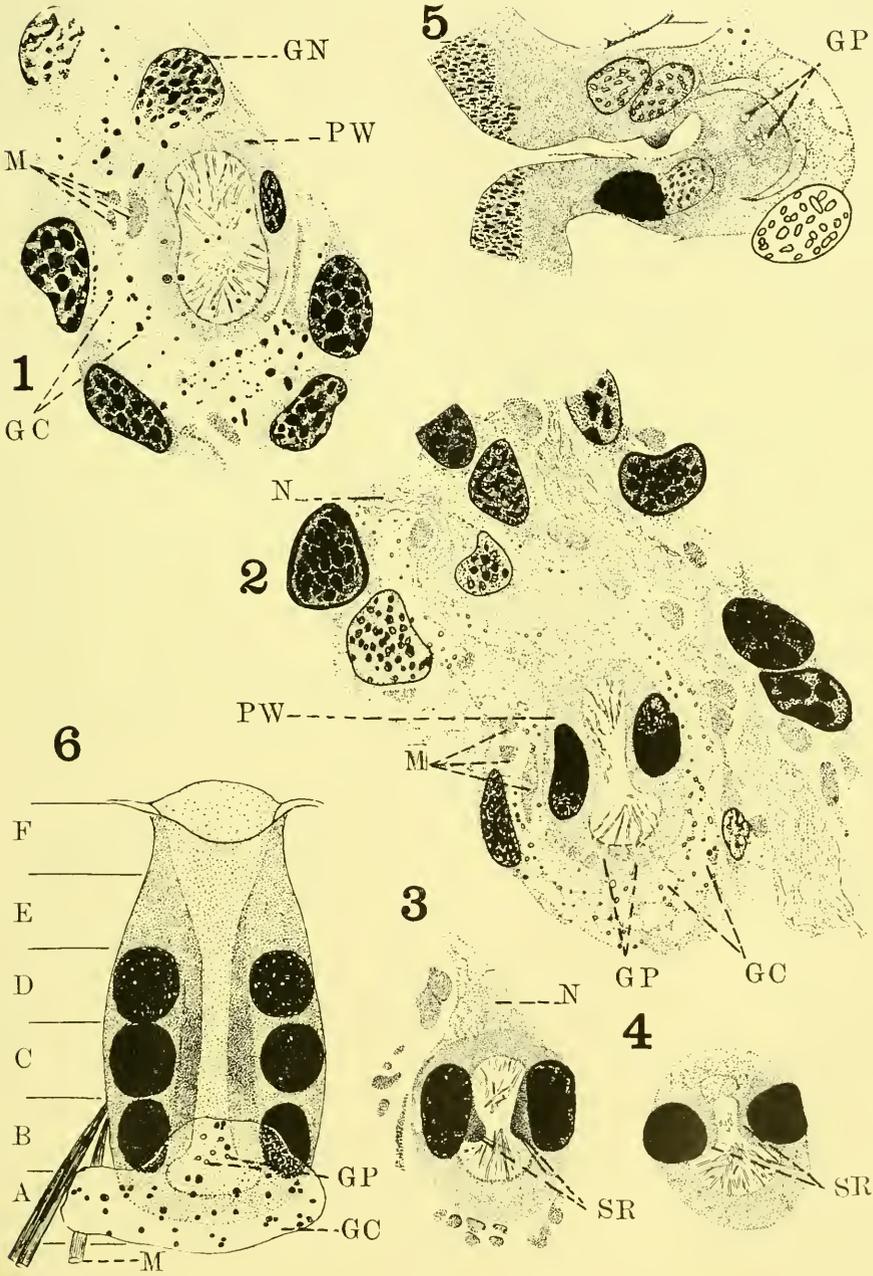
7 Reconstruction, based upon sections figured at 1, 2, 3, and 4. Right aspect of right pit. GC, gland cell with its nucleus; GP, pores of gland cell; M, muscles of pit; N, nerve of ciliated pit; G indicates the approximate level or plane of section shown in figure 5. $\times$ 2000.

8 Transverse section through dorsal ganglion, DGL. N, dorsal ganglion cells; Ph, wall of pharyngeal sheath; Mes, cells of mesenchyme; NVC, nucleus of visual cell; C, lateral cytoplasm of visual cell; L, lens-like cytoplasmic region of visual cell; Rh, cone-shaped rhabdome of visual cell; NAC, nucleus of accessory, pigmented cell of eye; Pg, region of laminated cytoplasm of accessory cell, which carries the pigment. $\times$ 1500.

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8

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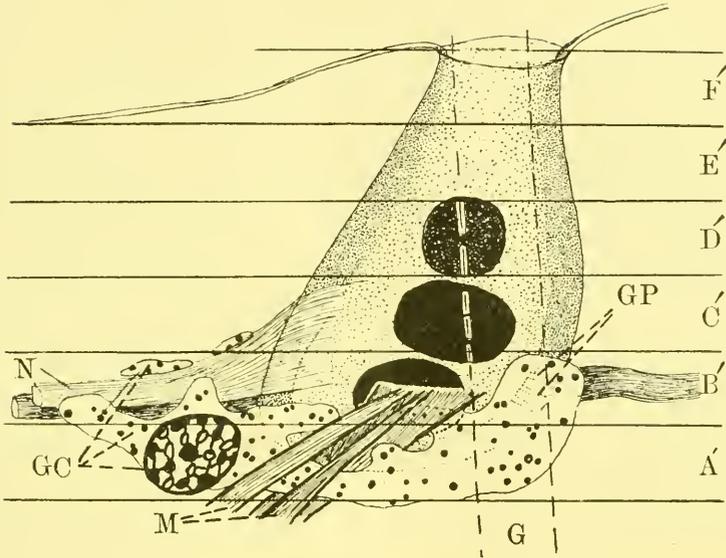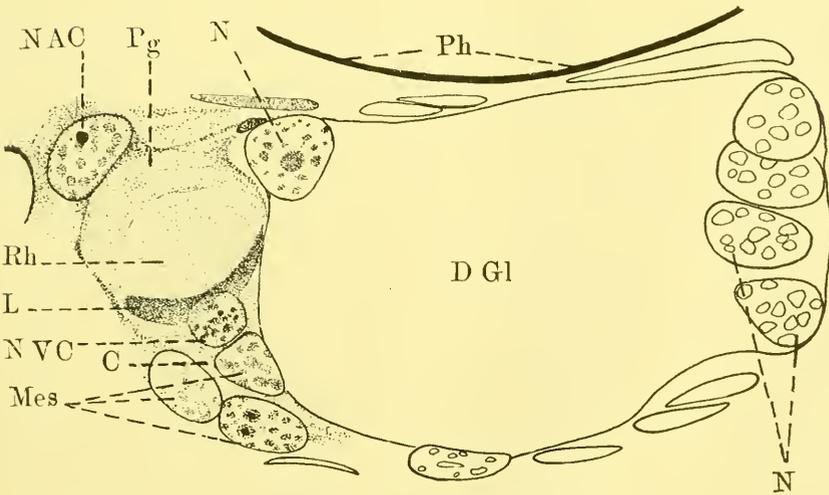
CHROMOSOME STUDIES

I. TAXONOMIC RELATIONSHIPS SHOWN IN THE CHROMOSOMES OF TETTIGIDAE AND ACRIDIDAE: V-SHAPED CHROMOSOMES AND THEIR SIGNIFICANCE IN ACRIDIDAE, LOCUSTIDAE, AND GRYLLIDAE: CHROMOSOMES AND VARIATION

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FOUR TEXT FIGURES AND TWENTY-SIX PLATES

CONTENTS

INTRODUCTION

In a study of the germ cells of the Tettigidae, concerned chiefly with the problem of synapsis, I have found such a surprising uniformity of numbers and size relations among the chromosomes throughout the eight species of the three different genera examined, that it seems advisable to present the evidence I have on the taxonomic value of the chromosomes and to make my observations on synapsis a second paper, Chromosome Studies. II.

For purpose of comparison with the Tettigidae, and also to emphasize the constancy of numbers and general size relations in three of the subfamilies of the Acrididae, I have worked out the complexes of Syrbula acuticornis Bruner, and of Chorthippus (Stenobothrus) curtipennis Harr., two species of the Truxalinae. Stenobothrus has been described by Davis ('08), Gerard ('09), and Meek ('10, '12) as having seventeen chromosomes. I believe, however, this genus in reality has twenty-three chromosomes, like other genera of the subfamily.

For the chromosome conditions in the Oedipodinae and Acridiinae, I have depended on the figures of Davis ('08).

In order to show that phenomena similar to those in Chorthippus may possibly occur in the Locustidae and Gryllidae, I have given figures from Buchner ('09), Davis ('08), and Baumgartner ('04) and from a paper by one of my students, Miss Carrie I. Woolsey. Here, again, I believe that the great amount of variation in chromosome numbers hitherto described may be due largely to the intimate association of certain chromosomes in some genera or species which in others may remain separate.

<div style="text-align:center">TAXONOMY</div>

According to Handlirsch ('08), the order Orthoptera includes five families, Acrididae, Locustidae, Gryllidae, Gryllotalpidae and Tridactylidae. Systematists divide Acrididae into nine subfamilies. Of these, five are found in North America: the Tettiginae (Tettigidae) (figs. 1–6, 9–13, 17, 19, 21), Eumastacinae, Truxalinae (figs. 7, 8), Oedopodinae (figs. 14, 18, 20, 22), and Acridiinae (figs. 15, 16 a, 16 b). Representatives of all but one have been used for chromosome study; in addition the Pamphaginae have been studied in Europe. In the present work I have studied more particularly the chromosomes of the first group. For reasons which will be stated further on, I rank the Tettiginae on a par with the Acrididae, rather than as one of its subfamilies, and shall therefore use for them the term *Tettigidae*.

1. Taxonomy and chromosomes

McClung ('05, '07) has advanced to a very considerable extent the contention, first advocated by himself, that a certain degree of parallelism exists between chromosome structure and those characters of the body which systematists recognize as of use in determining the relationships of species, genera, and families. He contends that the degree of relationship may be recognized just as precisely in germ-cell structure as in any other part of the body. As the number of species of Orthop-

tera examined for chromosome conditions increases from year to year, the results tend to confirm the correctness of this view. In the chromosomes of Orthoptera we seem to be dealing with morphological structures possessing a definite relative size and constitution as permanent as the cell itself. Their size relations and their behavior are probably constant, not only for all cells of an individual, but for every individual of the species. These size relations may vary, it is true, to a slight extent in the different species of a genus, as would be expected, and still more when the species of different genera are compared; but they may be reasonably constant within these limits for the subfamily, and, in many cases, for the family. Thus the degree of relationship is expressed as accurately in the nucleus as in any of the external body characters. A distinguishing feature in a germ-cell is, however, of much more importance than any character of the rest of the body, since it is located in a structure which may be considered the starting point of a new organism, and accordingly has an important rôle during the development of the body in the formation of *all* distinguishing characteristics of the individual—thus influencing not one part or organ, but many.

McClung held that for the Acrididae there are twenty-three chromosomes $(2N-1)$[1] in the male and for the Locustidae and Gryllidae each thirty-three. He did not make the count in the female, which has since been found to be $2N$; i.e., one more than in the male. So far as the Acrididae are concerned, these numbers will have to be modified to this extent. The subfamily Pamphaginae has 19 (σ) and 20 ($\circ$) (Granata, '10). This is a decided exception. Possibly a multiple chromosome may be involved, as in some Truxalinae and Acridiinae. Barring the Pamphaginae, McClung was correct, for the Tettigidae (figs. 1–6, 9–13), which have 13 (σ) and 14 ($\circ$) as the $2N-1$ and $2N$ numbers respectively, are possibly not related to the Acrididae as a subfamily (Tettiginae) and so might be expected to have peculiar chromosome conditions.

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[1] The designation N is used to indicate single or haploid number of chromosomes, $2N$ thus being the formula for the diploid or somatic number.

The fundamental number in the Locustidae seems to be some-what in doubt. Miss Stevens ('12) found thirty-seven in Ceuthophilus. In Jamaicana subguttata, unicolor, and flava (Woolsey, '15) the number is thirty-five. McClung ('02, '08) and Otte ('07) found the number in five genera to be thirty-three. Buchner ('09) found thirty-one in Decticus. Other cases (Steiroxys, Davis, '08) may be shown to have thirty-one, owing to the presence of compound chromosomes. The numbers most frequently found seem to be thirty-three and thirty-one. The extremely large number in Ceuthophilus may indicate a sub-family difference; in Jamaicana, a tribal difference.

Opinion as to the number of chromosomes in Gryllidae is un-settled. The most frequent is thirty-one or thirty-three ($\circ\!\!\!\!\!\!\nearrow$). It is quite possible that in Gryllidae also occur cases of com-pound chromosomes, since eight or ten **V**'s are present in species with low numbers (Baumgartner, '04; Gutherz, '09; Payne, '12). The tree-crickets (Baumgartner, '11) have 13 ($\circ\!\!\!\!\!\!\nearrow$) and 14 ($\female$); Stenopelmatus (Stevens, '05), forty-six. Such large variations as the last may indicate wide taxonomic difference.

2. Taxonomic characters compared

Before describing chromosomes, it is best to compare taxo-nomic characters of Tettigidae with those of three subfamilies of the Acrididae, also the characters of the genera and species I have studied within the Tettigidae.

The Tettigidae are more distinctly marked off from the subfamilies of Acrididae than these are from one another (com-pare figs. 1–6, 9–13 with figs. 7, 8, 14–16). The dividing line is not clearly marked between the Oedipodinae (fig. 14) and the Truxalinae (figs. 7, 8), and is but slightly more marked between either of these and the Acridiinae (fig. 15).

The chief distinguishing characters of Tettigidae are the extremely developed apical process of the pronotum (figs. 1–6, 9), the absence of pulvillus (fig. 12), and extreme reduction or ab-sence of elytra (ely., in figs. 1, 3, 4, etc.). Over against this in the subfamilies of the Acrididae the pulvillus is present (fig.

16), pronotum not extended over the abdomen (figs. 7, 8, 14–16, *ms'thx.*), and elytra are present and elongated (figs. 7, 8, 14–16, *ely.*) except in cases of brachypterism.   It is easy to see the much closer resemblance in all these characters among the subfamilies represented in figures 7, 8, and 15 than between any of these and the Tettigidae.   Thus, on the basis of these body characters the Tettigidae are clearly marked off from the Acrididae.

Internal structures present equally distinguishable characters. No organs illustrate this better than the gastric caeca.   In the Acrididae the five caeca (fig. 18) have each a large anterior and a smaller posterior extension (pouch).   The crop (fig. 18, *iglv.*) is relatively larger and more rectangular anteriorly than in the Tettigidae (fig. 17).   In the latter the caeca have only an anterior prolongation.   There are, however, two circular ridges, which may possibly serve for posterior caecal pouches.

In the reproductive organs also are characters distinguishing the two groups.   In the usual acrididean testis the follicles are long, sac-like structures joining the vas deferens close together (fig. 22).   This gives the testis a tassel-like appearance. Figure 22 is of Dissosteira carolina, an oedopodine, and is representative also of the Truxalinae and Acridiinae.   In the Tettigidae the plan of structure is more primitive.   The follicles are connected with the vas deferens at considerable distances apart in regular order (fig. 21), as in the typical acrididean ovary, except that ovarioles are not nearly so numerous as testicular follicles.

In histological characters there are also the same two types. The appearance of the tettigidean testicular follicle in longitudinal section (fig. 19) differs much from that of the Acrididae (fig. 20).   It may even be said to look more like that of Gryllidae or Locustidae.   The cell cysts extend completely across the follicle (fig. 19).   This is not true of the Acrididae (fig. 20), but is true of Gryllidae and Locustidae.   The cytoplasm of spermatogonial and spermatocyte cells is more compact, and the meshes of the cytoplasmic network are much smaller than in the Acrididae.   This is also a gryllid character.   These facts

do not necessarily indicate close relationship with Locustidae and Gryllidae, but they do show the wide divergence of the Tettigidae in these respects from what we find commonly in the subfamilies of Acrididae.

Upon the basis of these visible external and the grosser internal characters, we would at once separate what was formerly known as the Acrididae into two groups, on the one hand the Tettigidae and on the other the Truxalinae, Oedopodinae, and Acridiinae.

The Tettigidae are in turn divided into four subfamilies, the Cladonotinae, Metrodorinae, Tettiginae, and Batrachidinae. Of these we have represented in the United States only the last two. Of the genera which I have studied, two (Acridium [Tettix] and Paratettix) belonging to the former group and one (Tettigidea) to the latter. We should, therefore, expect to find among these three genera two more nearly related to each other in chromosome characters, than either of them is to the third. Before taking up chromosome characters, we shall consider the body characters.

The subfamilies Tettiginae and Batrachidinae may be distinguished from each other by the following points:

Tettiginae

1. Anterior femora compressed, carinate above.
2. Vertex, viewed from the dorsal side (figs. 3, 4, 5b, 13a), extends either scarcely to, or slightly beyond, the eyes.
3. Antennae composed of twelve to fourteen segments.
4. Pronotum in front truncate (figs. 1b, 2b, 3, 4, 5b, 6).

5. Dorsum flat, may be at same time slightly carinate or cristate. Median carina inconspicuous.
6. Humeral angles of pronotum obtuse (fig. 3, *ang. hum.*).

Batrachidinae

1. Anterior femora broadly sulcate above.
2. Vertex large and projects much in front of the eyes (figs. 10, 11).

3. Antennae composed of twenty-two segments (in Tettigidea).
4. Pronotum in front not truncate, but produced above the head; anterior dorsal margin angulate (figs. 10, 11).

5. Dorsum not flat, but obtusely tectiform. Median carina conspicuous.
6. Humeral angles of pronotum angulate.

7. Lateral lobes rounded, not angular (figs. 3, 5, *lob.1*).

7. Lateral lobes angulate (fig. 9, *lob. 1*)·

8. Eyes rounded rectangular, long diameters almost parallel (figs. 3, 4, 13a, 13b).

8. Eyes elongate-triangular (figs. 10, 11), long diameters forming an angle of 90° (figs. 10, 11).

9. No supra-ocular lobe of vertex extending over eyes.

9. Eyes partly covered by small supra-ocular lobes of vertex (figs. 10, 11, *lob. su'oc.*).

Within the subfamily Tettiginae the genera Acridium and Paratettix differ in the following respects:

| *Acridium (Tettix)* | *Paratettix* |

1. Vertex much broader than one of the eyes, projects beyond them (figs. 3, 4), angulate anteriorly.

1. Vertex as narrow as, or very slightly wider than, one of the eyes (figs. 13a, 13b), does not project beyond, truncate anteriorly.

2. Eyes small.

2. Eyes large, bead-like.

3. Pronotum does not project far overhead, does not reach posterior margin of eyes (figs. 3, 4).

3. Pronotum projects far overhead, reaches posterior margin of eyes (figs. 5b, 6, 13a, 13b).

4. Anterior margin of pronotum slightly angulate.

4. Anterior margin of pronotum decidedly truncate (figs. 5b, 6).

5. Body between shoulders not so wide as in Paratettix.

5. Body between shoulders wide (figs. 5b, 6).

Within Acridium (Tettix) I have studied four species. As in the case of the genera compared above, two of these species are more closely related to each other than either of them is to the third, obscurus. Of ornatus I have not a sufficient number of drawn cells to justify comparison of body characters with those of the other species. (See opposite page.)

By studying these characters it will be seen that granulatus and incurvatus usually agree more closely with each other than with obscurus.

THE CHROMOSOMES

Within the cell, the varying degrees of relationship which we have been looking at from a taxonomic point of view are again shown to a surprising extent by chromosomes. This is found in the constancy of numbers and in differences between size

	ACRIDIUM GRANULATUS (Figs. 1-3) (a)	ACRIDIUM INCURVATUS (fig. 4) (b)	ACRIDIUM OBSCURUS (figs. 2a, 2b) (c)
1. Integument.	Granulate or little rugose.	Granulate.	Arenose.
2. Vertex viewed from above	Nearly twice as wide as one eye (figs. 2b, 3).	Nearly twice as wide as one eye.	Fully twice width one eye, little more depressed than (a) and (b).
Front margin	Obtuse angulate.	Obtuse angulate.	Sub-truncate or scarcely convex.
Advanced beyond eyes	Considerably.	Considerably.	Little.
Occiput	Naked (fig. 3).	More covered.	Naked.
Crown of head in profile.	Above.	Above.	Level with superior margin of eyes.
3. Frontal costa advanced beyond eyes	Strongly.	Strongly.	Only one-fourth diameter of eye. At junction with median carina of vertex projecting as angulate eminence with apex.
Apex	Quite acute (figs. 1a, 3).	Acute.	Obtuse.
4. Pronotum, anteriorly	Truncate.	Truncate.	Truncate, strongly constricted before shoulders.
Posteriorly	Long extenuate; apex acute (fig. 1b).	More or less abbreviated.	Long; not so acute.
Antero-dorsal margin	Indistinctly obtuse, angulate.	Indistinctly obtuse, angulate.	Truncate.
Dorsum, transversely between shoulders	Narrow.	Wider.	Moderately broad.
Median carina	Tectiform. Distinctly elevated percurrent.	Wider, more tectiform. Distinctly elevated percurrent.	Flattened or sub-convex. Not elevated.
In profile	Nearly straight (figs. 1a, 1b, 3).	Arched (not straight).	
Humeral angles			Much more strongly prominent.
5. Eyes			
Viewed from above	Small.	Small.	Moderately large.
	Elliptic.	Elliptic, kidney-shaped.	Especially prominent.
Longitudinal axes	Parallel.	Not parallel.	
6. Antennae	Short, stout.	Short, stout.	Slender, long.

gradations. The differences depend upon degree of remoteness in relationship.

First of all, our short-horn grasshoppers may be divided into two large groups on the basis of number of chromosomes: the family Tettigidae having 13 ($\male$) and 14 ($\female$), and the family Acrididae with its three subfamilies having 23 ($\male$) and 24 ($\female$).

Within each of these groups the chromosomes present certain general size relations, which are fairly constant, but there are minor variations. The larger variations are found between different genera. More distantly related genera are less likely to be similar. The smaller variations between species of a genus, though more difficult to recognize, follow the same principle. The greater are likely to be found between more distantly related species, the lesser between those more closely related. This does not mean that more distantly related species may not occasionally be found with chromosomes more nearly alike than those of less distantly related species. Observations have not been extended far enough to determine that point.

1. Tettigidae

1. Acridium granulatus Scudd. a. Chromosomes of the male germ cells. Spermatogonial and somatic chromosomes of this, and of all other species of the family thus far studied, are of the rod-shaped type, and the spindle fibers are attached at the proximal end; i.e., the end which points toward the center of the cell plate at the time of cell division (figs. 23–28). Great extremes in the relative sizes of different chromosome pairs are characteristic of all the Tettigidae, but are most marked in this genus. Compare chromosomes 1, 1 or 2, 2 with 6, 6 (figs. 23–28). The chromosomes may be readily arranged into a series of six pairs besides the accessory chromosome. The latter is so nearly the size of the smallest pair that it would be difficult to distinguish between them, were it not that frequently the sex chromosomes show the 'woolly' surface texture (no. $2x$ in figs. 23, 25, 26, 27) often seen in other acrididaean spermatogonial stages (Sutton, '02; McClung, '05; Pinney, '08). Of

these six pairs, two (nos. 6, 6 and 5, 5) are more than twice the length of any other pairs. Nos. 4, 4 and 3, 3 are the intermediate pairs, the larger being about one-half that of no. 5 and less than one-half that of no. 6. Nos. 1, 1 and 2, 2, the smallest pairs, are easily distinguishable from nos. 3, 3 and 4, 4 and usually from each other. The sex chromosome is designated by $2x$ in this species because it ranks second in the total series. It is distinguished from the ordinary chromosomes numbered 2 by the addition of x. In general we have among the ordinary chromosome pairs three distinguishable size-groups: one embracing the largest (nos. 6 and 5), another the intermediate nos. 4 and 3), and a third the smallest (nos. 2 and 1). These three groups may be recognized in all the species of the family here studied. There is noticeable, as the measurements will later indicate, considerable difference in size between the two largest pairs, very little between nos. 4 and 3, but again quite an appreciable difference between nos. 2 and 1.

The size relations are more evident, though not as accurately shown, in the maturation divisions, and are best seen in views of division figures perpendicular to the axis of the spindle. In the Tettigidae the first maturation division is reductional. The members of each pair of chromosomes appear on the first maturation spindle attached to each other by distal ends only. This gives, exclusive of the accessory chromosome, six rods somewhat constricted, or even pulled apart, in the middle (figs. 29–34). Here again the six pairs may be grouped into three sizes, the extremely large (6 and 5), the intermediate (4 and 3), and the smallest (2 and 1).

The inequality in the size of 6 and 5 is well marked, shown by the 5's usually being more advanced in the act of separation than the 6's (figs. 29, 30, 31, 33). This character holds for the species of Acridium in distinction from those of Tettigidea, where it is only slightly marked. It also holds in Parattettix, though not to so great a degree.

The 3's and 4's are more nearly alike in size than the 5's and 6's, sometimes hardly distinguishable (figs. 30, 32, 34). The pairs of the smallest group are unequal in size, though frequently

it is difficult to distinguish them. In size the sex chromosome falls between 1 and 2, often so nearly like 1 that it is a question which to call it, $1x$ or $2x$. This, again, is a character of the genus Acridium. The sex chromosome passes undivided to one pole of the nucleus in the first maturation division. It always lies outside the plate (figs. 35, 36).

At the second division (figs. 37, 38) the inequality in the size of the two largest chromosomes is especially marked and the size relations of all are clearly shown.

b. *Chromosomes of the male somatic cells.* The same size relations appear in somatic cells though it is more difficult to pick out the members of the smallest and intermediate pairs. In animals taken before the last moult, dividing cells in large numbers were found in the following organs: mesenteron, proctodaeum, hypodermis, fat body, follicles of testis and ovary, and possibly in intestinal muscles. Of these tissues the columnar epithelium of the mesenteron showed the clearest cases of mitosis (figs. 39, 40). The cells were too long to be shown complete; accordingly only that portion which contains the dividing chromosomes was drawn. In figure 40 the two large pairs and their characteristic inequality are especially evident. One of the 4's and one of the 3's are seen foreshortened. These cells are of entodermal origin and their chromosomes resemble very much those of spermatogonia and oogonia.

Figures 41 and 43 show cells from the posterior part of the alimentary canal, probably of ectodermal origin. The size relations of the chromosomes are the same as before.

Figures 44, 45, and 47 represent cells from the hypodermis, the layer which secretes the cuticula. These cells contain pigment granules, which probably bear an important relation to the pigmentation of the cuticula. The number of chromosomes and the size relations are the same as in other tissues. The chromosomes are much shortened and thickened and have a tendency to clump together more than usual.

Figures 48a, 48b, 50a, and 50b are possibly nuclei of fibers from the outer muscular wall of the intestine. The number of chromosomes and the size relations are the same as usual. Fig-

ures 46 and 49 are of cells from the fat body; 49 of a normal cell, but 46 of a giant cell, in which were found not thirteen but twenty-six chromosomes. The fact that there were twenty-six chromosomes and that among these there were four of each of the six sizes indicates that this is probably a double cell with a double set of cell organs. It may have arisen by fusion of two cells, or by failure of the cell to divide after the nucleus had divided. Cells with multiple numbers of chromosomes occur frequently in this tissue.

c. Chromosomes in the female. Oogonial or oocyte divisions were not obtained, owing to incompleteness of material, but abundant mitoses were found in the walls of ovarioles. In every case they showed fourteen chromosomes. The x chromosome was distinguishable in over-decolorized cells by its 'woolly' appearance (figs. 51, 53, 55, 58), as was true in spermatogonia.

At this point it will be well to explain the Tables (I–XX) of chromosome lengths (pp. 193–196), prepared for comparison of the several chromosomes in the following genera and species. These show the relative lengths of the six autochromosomes and the relation that the length of the sex chromosome bears to their combined length. In preparing the tables the length of the image of each chromosome, as drawn at 3900 diameters magnification, was measured in millimeters. The measurement from the longer member of each pair was used in most cases, since it was evident, by focusing carefully, that apparent difference in length of the members of a pair was due to a fore-shortening of one of the members. When a chromosome appeared in end view, of course its length could not be obtained, and either the chromosome or the whole cell had to be discarded.

The chromosomes of somatic cells, spermatogonia, oogonia, and second spermatocytes are rod-shaped and may be measured with little difficulty. Those of first spermatocytes are either cross-shaped or rod-shaped. Here the members of pairs are joined at their distal ends and pulled out in opposite directions at their proximal ends, giving a cross or rod; in the latter the chromosome has double the length of a spermatogonial chromosome. To allow a comparison of spermatogonial and somatic

rod-shaped chromosomes with those of the first spermatocyte, all measurements of the former have been doubled. Most dependence is to be placed upon results from simple rod-shaped chromosomes of spermatogonia and second spermatocytes. The double chromosomes in the first spermatocyte divisions are less reliable (figs. 29, 31, 33, etc.), because the members of some pairs are more completely separated than others, which affects unequally the apparent lengths of the chromosomes. The diameters also vary. For this reason length measurements do not always accurately represent the size of chromosomes. But the differences in size are so evident to the eye that it is not difficult to decide where each chromosome belongs in the series.

To find the 'relative lengths' of the chromosomes, the actual length of each of the six pairs of autosomes was found for all available cells. Then the average length of all the chromosomes in each group (no. 1, no. 2, etc.) was found and the sum of these six averages, for the six groups of autochromosomes, was taken as 100 per cent, this being the average total length of the autosomes in all the cells of a given class studied. The 'relative length' of any given chromosome was found by comparing its length with the average total length of all six autosomes and is expressed in per cent. The sex chromosome was not included in making up the 100 per cent, but the percentage relation that it bore to the average sum of the autosomes was found in each case. It was kept separate from the other chromosomes because of its great variation in size in different genera, which ranged from no. 1 to no. 5. The autosomes were more uniform in size in this respect and afforded, therefore, a more suitable basis for comparison.

Explanation of Tables I to XX

The tables show by horizontal bars the average relative lengths of the six autosomes and the relative length that the sex chromosome bears to these in germ cells and in somatic cells for each species of Tettigidae studied. Measurements were made from camera lucida drawings of cells illustrated in the plates and from additional cells, not drawn. The intended length of each bar is indicated in millimeters at the right of the bar. The upper edge of the bar representing chromosome 6 is marked at centimeter intervals in each table. Consult also the text for further explanations.

ACRIDIUM GRANULATUS

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = ×3900	RELATIVE LENGTHS	mm.
			TABLE I — *Spermatogonia*	
23, 24, 25, 27, 28	1	12.26	10.48	29.7
	2	13.44	11.49	32.5
	3	15.56	13.31	37.6
	4	16.46	14.08	39.9
	5	26.28	22.48	63.7
	6	32.9	28.14	79.6
	2×	13.98	11.95	34.0
			TABLE II — *Second spermatocytes*	
37, 38	1	10.05	11.03	31.0
	2	10.25	11.25	32.0
	3	12.25	13.44	38.0
	4	13.33	14.63	41.4
	5	19.2	21.08	59.8
	6	26.0	28.54	80.8
	2×	10.13	11.1	31.5
			TABLE III — *Spermatogonia and second spermatocytes*	
	1		10.6	30.0
	2		11.41	32.3
	3		13.33	37.7
	4		14.24	40.2
	5		22.15	62.9
	6		28.24	79.9
	2×		11.77	32.6
			TABLE IV — *Follicle cells of female*	
51, 54, 55, 57, 56, 58	1	12.8	10.28	29.2
	2	15.2	12.21	34.6
	3	17.27	13.89	39.4
	4	18.98	15.26	43.4
	5	26.45	21.20	60.1
	6	33.83	27.14	76.8
	2×	13.05	10.48	29.8
			TABLE V — *Average of all except first spermatocytes*	
	1	11.7	10.4	29.5
	2	13.33	11.89	33.7
	3	15.14	13.50	38.3
	4	16.38	14.61	41.4
	5	24.48	21.83	61.8
	6	31.06	27.70	78.5
	2×	12.64	11.27	32.0
			TABLE VI — *First spermatocytes*	
29–33	1	10.7	11.07	31.4
	2	11.9	12.3	34.9
	3	13.7	14.18	40.2
	4	14.9	15.42	43.6
	5	21.1	21.84	61.8
	6	24.3	25.15	71.4
	2×	13.0	13.45	38.6

Bar-chart scale: 1 2 3 4 5 6 7 8 cm.

ACRIDIUM INCURVATUS

TABLE VII — *First spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. $= \times 3900$	RELATIVE LENGTHS	mm.
61–64	1	13.3	11.0	31.0
	2	14.0	11.6	32.9
	3	16.2	13.4	38.0
	4	17.3	14.3	40.5
	5	27.2	22.5	63.8
	6	32.8	27.15	77.1
	1×	13.3	11.0	31.0

ACRIDIUM ORNATUS

TABLE VIII — *First spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. $= \times 3900$	RELATIVE LENGTHS	mm.
65–66	1	18.0	8.55	24.3
	2	25.1	11.92	33.7
	3	30.8	14.63	41.4
	4	33.4	15.86	45.1
	5	45.2	21.47	60.9
	6	58.0	27.55	78.2
	1×	16.7	7.9	22.4

ACRIDIUM OBSCURUS

TABLE IX — *Spermatogonia*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. $= \times 3900$	RELATIVE LENGTHS	mm.
67–68	1	14.6	10.0	28.3
	2	16.2	11.1	31.5
	3	19.4	13.3	37.7
	4	20.5	14.1	40.0
	5	32.1	22.1	62.6
	6	42.8	29.4	83.3
	1×	12.6	8.7	24.7

TABLE X — *First spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. $= \times 3900$	RELATIVE LENGTHS	mm.
69, 77	1	9.63	10.43	29.5
	2	10.33	11.19	31.7
	3	12.5	13.32	37.7
	4	13.46	14.58	41.3
	5	19.08	20.67	58.6
	6	27.33	29.60	83.9
	1×	11.16	12.09	34.3

PARATETTIX CUCCULATUS

TABLE XI — *Spermatogonia*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. $= \times 3900$	RELATIVE LENGTHS	mm.
78–82	1	13.58	11.4	32.3
	2	14.98	12.6	35.7
	3	17.62	14.8	41.9
	4	18.9	15.9	45.0
	5	26.1	22.0	62.3
	6	28.0	23.6	66.8
	3×	16.5	13.9	39.4

PARATETTIX COCCULATUS (Continued)

TABLE XII *First spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = ×3900	RELATIVE LENGTHS		mm.
83, 84 and three others	1	7.47	12.2		34.6
	2	8.43	13.8		39.1
	3	8.9	14.6		41.4
	4	9.75	15.9		45.1
	5	12.5	20.4		57.8
	6	14.1	23.0		65.2
	3×	10.52	17.2		48.8

PARATETTIX TEXANA

TABLE XIII *Second spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = ×3900	RELATIVE LENGTHS		mm.
90 + others	1	8.4	11.4		32.3
	2	9.1	12.4		35.1
	3	11.2	15.2		43.1
	4	12.0	16.3		46.2
	5	15.4	20.9		59.2
	6	17.4	23.7		67.2
	3×	9.5	12.9		36.6

TABLE XIV *First spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = ×3900	RELATIVE LENGTHS		mm.
85, 87, 89	1	10.1	13.3		37.7
	2	10.6	14.0		39.7
	3	11.3	14.9		42.2
	4	11.7	15.4		43.6
	5	15.1	19.9		56.4
	6	17.0	22.4		63.5
	3×	13.5	17.8		50.4

TETTIGIDEA PARVIPENNIS PENNATA

TABLE XV *Spermatogonia*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = ×3900	RELATIVE LENGTHS		mm.
91	1		11.6		32.9
	2		12.9		36.6
	3		14.2		40.2
	4		14.3		40.5
	5		22.3		63.2
	6		24.6		69.7
	5×		15.3		43.4

TABLE XVI *First spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = ×3900	RELATIVE LENGTHS		mm.
94, 99	1		11.2		31.7
	2		12.5		35.4
	3		13.7		38.8
	4		14.9		42.2
	5		21.95		62.5
	6		25.6		72.5
	5×		16.3		46.2

TETTIGIDEA PARVIPENNIS

TABLE XVII — *Spermatogonia and secondary spermatocyte*

FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = × 3900	RELATIVE LENGTHS	mm.
104, 105, 107, 122, 124, 125	1	10.13	10.49	29.8
	2	10.8	11.19	31.7
	3	13.23	13.71	38.8
	4	14.88	15.42	43.6
	5	23.16	24.0	68.0
	6	24.3	25.18	71.4
	5×	17.57	18.2	51.6
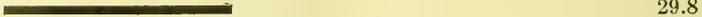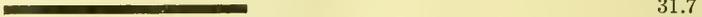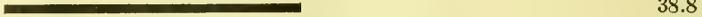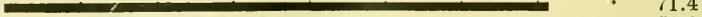
TABLE XVIII — *Fat body*
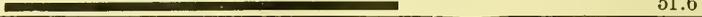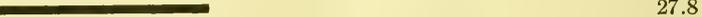
FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = × 3900	RELATIVE LENGTHS	mm.
108–110	1	11.7	9.8	27.8
	2	13.7	11.5	32.6
	3	15.8	13.3	37.4
	4	18.6	15.6	44.2
	5	28.7	24.2	68.6
	6	30.5	25.7	72.8
	5×	19.7	16.6	47.0
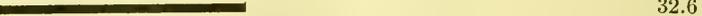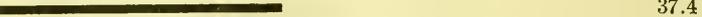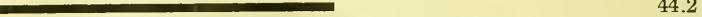
TABLE XIX — *First spermatocyte*
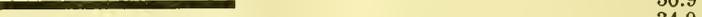
FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = × 3900	RELATIVE LENGTHS	mm.
111–119, 121	1	11.7	10.9	30.9
	2	13.2	12.3	34.9
	3	14.6	13.6	38.5
	4	16.0	14.9	42.2
	5	25.0	23.3	66.0
	6	27.1	25.2	71.4
	5×	20.5	19.1	54.1
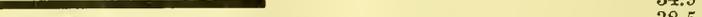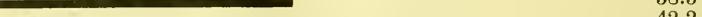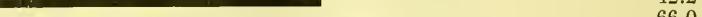
TABLE XX — *Oögonia and follicle cells, (♀)*
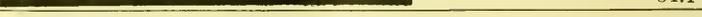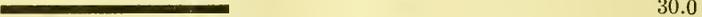
FIGURES	NUMBER OF THE CHROMOSOMES	LENGTH IN MM. = × 3900	RELATIVE LENGTHS	mm.
101–103	1	15.6	10.6	30.0
	2	17.0	11.6	32.8
	3	19.9	13.6	38.5
	4	22.6	15.4	43.6
	5	32.4	22.1	62.6?
	6	39.6	27.0	76.5?
	5×	26.1	17.8	50.4
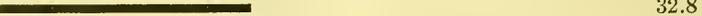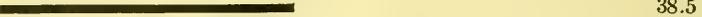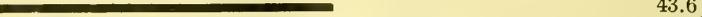
In the tables giving first spermatocyte lengths the sex chromosome appears longer than in the tables of other cells. This is due to the peculiar behavior of the sex chromosome in these cells, where it is relatively longer and narrower than in other kinds of cells. Its length according to the diagram is therefore greater than it should be.

An examination of the tables will show considerable variation in the length of each chromosome for cells of different tissues. Some of this variation, no doubt, is due to faults in the measurement or drawing of the chromosomes, as well as to cases of fore-

shortening. The results are, therefore, not so satisfactory as desired. Nevertheless, there is a certain amount of agreement in the results which cannot be overlooked; it is fundamental and of use in comparing genera and tribes.

The arranging of the autosomes in three groups—nos. 1 and 2, nos. 3 and 4, and nos. 5 and 6—is best brought out in the tables of spermatogonia and second spermatocytes. The extreme inequality of the two largest pairs may also be seen in the same tables. There is one exception to this; viz., in the tables of first spermatocytes. An examination of the figures of the cells used, however, will show that this is due to variation in the thickness of the chromosomes not expressed in the length measurement. The small size of the 'accessory,' which ranks between nos. 1 and 2 or close to no. 2, is evident from Table V of the average of rod-shaped chromosomes. It is much too long, as already explained, in the first spermatocyte cells.

The slenderness of the sex chromosomes in the follicle cells of the ovary (Table IV) is noticeable. They are 'woolly' and smaller than usual. There may be some connection here with their duality in the female sex.

d. Summary for Acridium granulatus. Acridium granulatus possesses the family number of chromosomes—six pairs and either one or two sex chromosomes, according to the sex—and shows a grouping of these pairs according to the size relations which are characteristic of the family: two extremely large pairs, two intermediate pairs, and two very small pairs. It further bears the subfamily characters: first, in having the sex chromosomes near to the smallest pair of the complex in size; secondly, in having a marked inequality between the two largest pairs; and thirdly, in having very small cells. It bears the generic character of small sex chromosomes and nearly an extreme of inequality between the two largest pairs. The chromosomal relations here summarized may be readily seen in Table I for rod-shaped chromosomes.

2. Acridium incurvatus Hanc. Acridium incurvatus (figs. 4, 59–64; Table VII) taxonomically is closely related to granulatus. The chromosomes also show this close relationship. It possesses

the number characteristic of the family—six pairs and one
(♂) or two (♀) sex chromosomes, the pairs being in three groups
according to size relations characteristic of the family. The sub-
family character is also shown by a sex chromosome that in size
is near to the smallest of the complex, by a marked inequality
between the two largest pairs, and by small cells. It has the
generic characters: nearly the extreme of inequality between
the two largest pairs and smallness of the sex chromosome.
These similarities serve to put incurvatus into the same family,
the same subfamily, and the same genus as granulatus.

According to the systematic descriptions of the species one
would expect the chromosomes to be more nearly alike in these
two species than in any others. The measurements are not
sufficiently accurate to be of use in specific relations. Attention,
however, should be called to the *differences* between the chromo-
somes of the two species.

The difference in length between the 5's and 6's of incurvatus
(22.50 to 27.15) is not quite as great as in granulatus (21.83 to
27.70). The gap between the 3's and 4's is only slightly less, being
13.40 to 14.30 compared with 14.18 to 15.42. The same may
be said of the smallest pairs (1's and 2's). There is a wider gap
between the smallest and the intermediate groups than in granu-
latus (Tables VI, VII). The sex chromosomes is slightly smaller
(11.95 to 13.45). Finally the cytoplasm of the cells is consider-
ably more dense in the former. These differences might be
spoken of as specific. However, they cannot yet be said to be
satisfactorily established.

The zygote number (2N) of chromosomes has been shown in
follicle-cell mitoses, no spermatogonial mitoses being available.
These mitoses show that numbers and size relations may be
readily followed even in somatic cells.

According to Table VII, the length ratios of the autosomes
in incurvatus are nearer to the average rod-shaped chromosomes
of granulatus (Table V) than to the first spermatocyte chromo-
somes (Table VI). This is due to the fact that the chromosomes
in the first spermatocyte of incurvatus (figs. 61–64) are still
in the late prophase, not pulled out unequally as in granulatus

(figs. 29–33). It is true that there is here some abnormal lengthening of some of the double chromosomes, as may be seen by comparing 5 and 6 of figure 62, but this is not so great as in some cases of first spermatocyte chromosomes in granulatus. It seems safe to say that there is a striking similarity in the chromosome ratios of the two species (compare Table VII with I and V), although there are enough differences to serve as a basis for specific distinction.

3. *Acridium ornatus Harris.* Only two cells (figs. 65, 66) from this species are shown. They are unfavorable for determining accurately the relative lengths of the chromosomes, since the latter are in prophase and differ in the degree of condensation to which each has advanced. This probably accounts for the extreme difference between the two smallest pairs (Table VIII, 1, 2). Chromosome no. 1 (figs. 65, 66) is much more contracted than either 2, 3, or 4. A similar precocious condensation is seen among the smaller tetrads in Syrbula and Chorthippus (figs. 149, 163). The sex chromosome is precociously condensed and therefore appears shorter (Table VIII) than it otherwise would be. Chromosomes 2, 3, and 4, on the contrary, appear more slender (fig. 66) than nos. 1, 5, and 6, and therefore relatively longer than they otherwise would be, while the latter are relatively shorter (Table VIII). In spite of these discrepancies, the sizes characteristics of the family, subfamily, and genus may be readily recognized. It seems justifiable to say that here, again, the ratios are sufficient to put this species into the genus Acridium rather than Paratettix or Tettigidea. The material is too scant to warrant going into specific differences.

4. *Acridium obscurus Hanc.* This species (figs. 2a, 2b, 67–77; Tables IX, X) is not so closely related to granulatus as is incurvatus. The germ cells of the male have thirteen chromosomes. I have not examined those of the female nor the somatic cells.

The chromosome size relations, as in ornatus and incurvatus are sufficiently similar to those of granulatus to place obscurus within the genus Acridium. They agree with the other species

(figs. 67–77; Tables IX, X) in having autosomes with a great range of sizes between the smallest and largest, an exceedingly large gap between 4's and 5's, a large difference between 5's and 6's, and a sex chromosome (1x) in length near the smallest autosome.

The differences from granulatus are greater than from incurvatus. The two largest pairs in obscurus differ more in length than in any other species of the genus. This is most evident in first spermatocytes, the lengths being respectively 20.67 and 29.60. This difference cannot be far from correct, since in spermatogonia the lengths are 22.1 to 29.4. The ratio of these chromosomes in the first spermatocytes of incurvatus is 22.5 to 27.15, and in granulatus, 21.84 to 25.15. The last number (25.15), however, is not normal, as comparison with the spermatogonial chromosomes (22.48 and 28.14) will show. Comparing measurements of the spermatocytes in incurvatus with those of the spermatogonia in granulatus and obscurus, it appears that incurvatus is more nearly like granulatus than is obscurus. In the last species the 6's are relatively the longest (29.4) and the gap between the 5's and 6's the largest yet found (figs. 69–77).

The intermediate pairs (3's and 4's) are almost indistinguishable from each other, especially in spermatogonia, though measurement shows them to be slightly different. In this respect they are similar to those of the other species of the genus. However, the difference in size between the no. 3's and the no. 2's is greater in obscurus than in granulatus or incurvatus (Tables I, VII, IX).

In the spermatogonia of obscurus the 2's are slightly larger than the 1's and the sex chromosome. But in the spermatocyte the sex chromosome appears larger than either 1's or 2's. This is due to the fact that in the spermatocyte the diameter of the sex chromosome is less than that of the other chromosomes and its length therefore greater than it should be. The more correct measurement is probably that of the spermatogonia, where the sex chromosome is the smallest of the series (figs. 67–70). For this reason it has been numbered 1x. It is 'woolly' in appearance (figs. 67–68). Here, again, is clear evidence of the 'differential chromosome' in spermatogonia.

Another difference shown by obscurus is that both soma and germ cells are smaller than in any other species studied; possibly a specific difference.

Obscurus shows conditions of chromosome size sufficient to place it in the genus Acridium; it differs from granulatus and incurvatus in having a greater difference of length between the 5's and 6's, a slightly wider gap between the 2's and 3's, a slightly smaller sex chromosome, and smaller cells.

In the spermatogonia of obscurus both resting and dividing nuclei (figs. 67, 68, c) frequently contain a spherical body (sometimes two) which stains like chromatin. At mitosis it passes undivided to but one of the daughter cells. Its presence in so many spermatogonia indicates that it may divide at some of the divisions or at intervening stages. However, when two are present, they are unequal in size. This suggests only transitory bodies. In both the prophase and metaphase of the first spermatocyte, from one to three unpaired bodies occur which may represent one or both spermatogonial bodies (figs. 69–74). One, of course, is the sex chromosome (fig. 69). Of the other two, one (con.) is small and loose; its staining capacity varies, though it never stains deeply, and frequently is almost indistinguishable. It is cone-shaped (figs. 69–74, con.), but sometimes appears partly divided (figs. 72, 73). A third one (c) of the unpaired bodies may be present in either nucleus or cytoplasm. It appears like a chromatoid nucleolus, and may be the sort of structure encountered in the spermatogonia. It is always spherical (figs. 73, 74) and passes undivided to one pole in the first maturation division. These structures behave somewhat like chromosomes in staining and in sometimes being drawn into the equatorial plate (figs. 72, 74) at division. It seems possible that the cone-like body (figs. 69–74, con.) may be a chromosome fragment, the result of an unequal division at some previous time.

5. *Paratettix cucculatus Morse.* In Paratettix cucculatus (figs. 78–84) the cells are decidedly smaller than in Acridium, and the histological appearance is different. In external characters the genus is nearer to Acridium than to Tettigidea, which will be taken up after Paratettix. Systematists recognize this fact by

placing Acridium and Parattetix in one subfamily and Tettigidea in another. These relationships are shown in the chromosomes, and in the size of cells. The 5's and 6's are very large and differ considerably in size. The inequality between them (22.0 to 23.6, Tables XI, XIII) is, however, not nearly so marked as in Acridium (22.48 to 28.14, Tables I, VIII). Again, the range of size from largest to smallest is not nearly so great as in Acridium (29.4 to 10.0, Table IX; compare also figs. 78 to 82 with 67, 68).

The sex chromosome in Paratettix cucculatus ($3x$) is slightly larger (13.9) than in Acridium (11.9). In length it comes nearest the 3's, so is designated by $3x$ instead of $1x$ or $2x$, as in Acridium. However, it is, like Acridium, near the size of the smallest group (1's and 2's). It may, as in Acridium, be distinguished in spermatogonia by its 'woolly' appearance (fig. 82). The measurement (17.2 is too high, as usual in first spermatocytes.

6. *Paratettix texanus Hanc.* This species (figs. 85–90)[2] is similar to cucculatus, but the range in size of chromosomes (first spermatocyte) is somewhat less. This may be due to the greater diameter and consequent shortening of the 5's and 6's. In the second spermatocyte the range is about the same as in the spermatogonia of cucculatus, though the 5's differ (22.0 in cucculatus; 20.9 in texanus).

The sex chromosome is about third in size and slightly larger than in the preceding genus. Its rank, according to length in first spermatocytes, is not correct, since it is longer and of less diameter, compared with autosomes, than it is in the spermatogonia or in the second spermatocytes. The measurements of first spermatocytes were, therefore, not used.

Paratettix is, therefore, distinguished from Acridium by smaller cells; by a less range in extreme lengths of chromosomes; by slightly larger sex chromosome; and by a smaller difference in the length of the two largest chromosomes.

7. *Tettigidea parvipennis pennata Morse.* Tettigidea is in body structure much farther from Acridium than is Paratettix, so far, in fact, that it is placed in a different subfamily, the Batra-

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[2] Figures 88 to 90 are from a slide of Paratettix texanus leucocephalus Nabours, kindly furnished me by Dr. Mary T. Harman.

chidinae. Cell structure and chromosomes likewise show this relation. The cell has a diameter one-fourth to one-third greater than that of Acridium or Paratettix. The network of the cytoplasm is much looser.

Figures 91 and 92 show thirteen chromosomes ($\male$), arranged as usual in six pairs plus the sex chromosomes: two large (6's and 5's), two intermediate (4's and 3's), and two small pairs (2's and 1's). The sex chromosome (5x) ranks fifth in size. The 5's and 6's are more nearly equal than was true in any of the other genera (Tables XV–XIX). Table XX (oogonia and follicle cells) shows a much greater difference between the 5's and 6's. Possibly some error may be the cause of this, as only three cells were used.

Figures 93 to 99, prophases and metaphases of the first spermatocyte, show the number and size relations more perfectly than the spermatogonia do. Figure 99 lacks a single pair of chromosomes (4's), due probably to loss in sectioning. It will be seen from these figures and Table XVI that a greater interval in size (8.6) occurs between the 5's and the 4's in Tettigidea than in Acridium (7.8) or Paratettix (5.1). Also the gap between the 2's and 3's is not so great as in these genera. In other words, the intervals which separate the four largest autosomes are more nearly equal in Tettigidea than in either of the other genera. These relations may be most readily appreciated from Tables I–XX. Figure 100 shows an oogonium. The number of chromosomes is, as usual, fourteen. Size relations are well shown.

8. *Tettigidea parvipennis Morse.* This species, in chromosomes, is so much like Tettigidea parvipennis pennata that it is impossible to tell them apart. Figures 101 to 103 are of oogonia. The number is fourteen (six pairs plus two sex chromosomes). The slight inequality of the two largest pairs (6's and 5's) is evident, especially in figure 101. In figures 102 and 103 no. 5 is foreshortened. This affects the results in Table XX. The 4's and 3's show the usual gradation in size.

Figures 104, 105, and 107 are spermatogonia with the usual thirteen chromosomes. In figure 106 a large chromosome (no.

6) is drawn from each of several cells showing the split and especially the large knobs, at the distal end, so common in this species. Sometimes the constriction is so great that one could imagine the knob to be a small attached chromosome. Is it possible that this knob corresponds to one of the smaller of the twenty-three or twenty-four chromosomes of the Acrididae? It might thus help to account for the smaller number, thirteen or fourteen, of the Tettigidae.

Figures 108 to 110 are of mitoses in male somatic cells from fat-body tissue. Here the chromosome numbers and their relative sizes are the same (Table XVIII) as in germ cells.

Figures 111 to 119, and 121 are metaphases of first spermatocytes showing number and size relations of chromosomes as before. Figure 121 lacks one pair (5's) due to sectioning. The slight inequality of 5's and 6's and the absence of as large a gap between the 2's and 3's as exists in Acridium and Paratettix are very evident.

Figures 122 and 123, representing two anaphase stages of the first spermatocyte, show that all chromosomes except $5x$ divide. This is probably the reduction division. The sex chromosome tends to drop behind the others in the latter part of its passage to the pole (fig. 123), though it starts ahead of them (fig. 122).

Figures 124 to 127 are metaphases of second spermatocytes. There are two sorts of cells, some containing seven, others only six chromosomes. The relative sizes (figs. 124, 125) are accurately shown. Nos. 5 and 6 are almost alike; $5x$ is clearly between 4 and 5. Further, 4 and 3 are nearly alike, as are also 1 and 2. One of the latter (no. 2), however, is always more slender than the other. This is seen even in first spermatocyte divisions, and it may be this autosome that condenses before the others in the first spermatocyte prophase stages (no. 2, figs. 65, 66). Figure 126 represents a cell sister to that seen in figure 127. These show that all chromosomes, including $5x$, are split in the second spermatocyte division.

The metaphases and anaphases of the first spermatocyte, shown in figures 128 to 135, are from young specimens belong-

ing to Tettigidea (probably parvipennis). They repeat and emphasize what has been found in parvipennis and p. pennata. In figures 132 and 134 tardy divisions of certain chromosomes are seen, which may be of significance in the question of reduction and Mendelian segregation, and may have some bearing upon the question of unequal tetrads. They demand further study.

9. *Unequal homologous chromosomes.* A female Acridium granulatus was found, in which there were five long chromosomes among the fourteen instead of the expected four. Later, the same condition was found in a male in first spermatocytes, where homologous chromosomes were separating from each other. This, fortunately, gave a clew as to what the long chromosomes paired with, for, with one exception, all the chromosomes were present, of normal size, and paired normally. The exception was in the no. 1's, which were represented by only a single member, and this paired with the fifth long chromosome (figs. 141–145). Anaphase figures (146, 147) showed this small chromosome separating from the abnormal mate (no. 1) and going to the opposite pole.

After seeing this condition of pairing in the chromosomes of the male, those of the abnormal female were easily paired as follows: two 6's, 5's, 4's, 3's, 2's, two 2x's, one no. 1, and the abnormally large no. 1. For the female the counts were made in the cells of the walls of ovarioles.

In the male individual the abnormal no. 1 shows a constriction (figs. 142, 144, 147) at a distance from its distal end equal to the length of the normal no. 1, with which it had been paired. This may possibly be the no. 1 portion of the abnormal chromosome. (See Chromosome Studies. III.)

The members of the unequal pair bear no constant relation to the sex chromosome in their distribution to either the second spermatocytes or the sperm cells, as figures 141–147. show. The abnormal chromosome (1) passes as frequently to the pole receiving the sex chromosome as to the pole lacking it. This has nothing to do with the unequal tetrad reported by Baumgartner ('11) for Gryllotalpa, for that unequal tetrad has since been shown by Payne ('12) to be a group of sex chromosomes.

In T. parvipennis one individual found showed the no. 4 chromosomes unequal. In all dividing first spermatocytes these (united) formed an unequal tetrad (figs. 115, 118, 119, 120, 122). I infer that the no. 4 chromosome, which came from one of the parents, was originally deficient in size. This character seems persistent enough to be preserved in all the cells of the first spermatocyte found dividing. Moreover, the same deficiency occurs in all the somatic cells studied—the fat-body cells (figs. 104, 105, 108).

The bearing which these unequal tetrads have upon questions of synapsis and reduction has been discussed in Study III, but will also be considered later in this paper.

2. Truxalinae (Acrididae)

In contrast to the family Tettigidae,[3] the three subfamilies, Truxalinae, Oedipodinae, and Acridiinae, of the Acrididae, have in general 23 (σ) and 24 (φ) chromosomes. Exceptions to these numbers have been found in Chorthippus (Stenobothrus) by Davis, Meek, and Gerard, and in Hesperotettix and Mermiria by McClung ('05). McClung found Hesperotettix to have 23 in the spermatogonia and 11 in the first spermatocyte, and Mermiria to have likewise 23 in the spermatogonia, but only 10 in the first spermatocyte. He believed that "the reason for the deviations is due to unusual associations of the spermatogonial chromosomes in the spermatocytes." I believe that likewise in Chorthippus we have not 17 chromosomes, but 23, and that here there is a *permanent* association of certain nonhomologous chromosomes, such as no. 11 with 7, no. 10 with 8, or no. 9 with 5, which are not associated in the majority of genera. To show how this may explain the peculiar numbers, I describe first the conditions in Syrbula acuticornis, an example of the 24–23 chromosome genera, in which no such association occurs.

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[3] The subfamily Tettiginae should be removed from the family Acrididae and raised to the rank of a family (Tettigidae) coördinate with the Acrididae.

1. *Syrbula acuticornis Bruner.* In the spermatogonia there are (fig. 148) twenty-three chromosomes, consisting of eleven pairs and the sex chromosome. I have numbered the autosomes in sequence, according to size, from smallest to largest: two 1's, two 2's, two 3's, etc. The sex chromosome ranks tenth from the smallest and is therefore marked $10x$. The 9's, 10's, and 11's are large. There is a considerable gap in size between the 8's and 9's. The 4's, 5's, 6's, 7's, and 8's form an almost uniformly graded series. A more considerable gap occurs between the 3's and 4's. The 1's, 2's, and 3's form the small series, although a much wider gap occurs between the 2's and 3's than between the 1's and 2's.

The chromosomes are all of the rod-shaped type and at metakinesis lie at right angles to the long axis of the spindle. In this plane they are arranged in radial fashion around the center of the plate (fig. 148), their pointed proximal ends, to which the spindle fibers attach, turned inward; the large blunt distal ends turned outward. It is important to note this arrangement in making comparison with what is found in Chorthippus.

I have drawn a series of stages from the prophase to the metaphase of the first maturation division. Figure 149 is of a nucleus coming out of parasynapsis. There are eleven first-spermatocyte autosomes, each split and more or less twisted spirally, and one sex chromosome ($10x$). The latter is also split and may be recognized by its condensed condition. The other chromosomes, except nos. 1 and 4, are alike in having reached about the same stage of unwinding and condensation. No. 1 is farther along than the others, and no. 4 exceeds no. 1, as indicated by its condensed condition, which approaches that of the sex chromosome. There is a difference between the ends of these conjugated chromosomes. One end usually has two dark knobs (x, x), from which the attraction fibers arise, whereas the other lacks any such knobs. These knobs are probably the 'polar granules' described by Pinney ('08).

In figures 150 to 152 a and 153 (later stages) the separation along the split, which was present in the spirally twisted chromosomes (fig. 149), is much more conspicuous. The spiral twist-

ing has disappeared, probably due to an unwinding process.
Each chromosome consists of four parts and may be considered
as being split in two longitudinal planes, one at right angles to
the other (figs. 151, 152 a–c, 154.)   This is more evident in some
chromosomes (fig. 149, nos. 1, 4) than in others (fig. 149, nos.
5, 10, 11), and usually more pronounced in later prophases (fig.
150) than in earlier ones (fig. 149).   Figure 154 represents
chromosome no. 4 in five stages of the process of splitting lead-
ing up to the metaphase condition.

In figure 155 the twelve chromosomes are shown in a stage
just preceding the breaking up of the nuclear wall.   The four
parts and the more or less characteristic shape for each chromo-
some (tetrad) are now evident.   No. 11, however, is not in its
typical form.   This may be seen in figures 156 d and 156 f.   Fig-
ures 156 a–f show a number of forms assumed by prophase chromo-
somes.   Their form depends much upon the length of the sper-
matogonial rods which conjugated to produce them.   Figures
156 a and 156 b are views of two conditions which are assumed
by a no. 1, 2, or 3 pair.   The attraction fibers probably arise
at x, x, for in the metaphase of this division such chromosomes
are rods, frequently constricted in the middle, to whose ends the
attraction fibers are attached.   During the preceding prophase
stages the chromosome has become split in one plane (fig. 156,
I, I) and the halves thus produced have separated at the end
which bears the knobs and have rotated around the opposite
end as a fixed center, each through an arc of 90° until the two
halves, still attached to each other at the end opposite the knobs,
form a nearly straight rod.   While this separation and rotation
of the halves resulting from the first split is going on, each half
begins to show its secondary split (II, II, fig. 156b), or else has
already acquired it before the rotation begins.   Without giving
my reasons here, I will simply say that I believe the first or pri-
mary split (I, I) produces the reductional division; i.e., separates
homologous spermatogonial chromosomes, and that the second
split (II, II) is an equational division of the now end-to-end
conjugated spermatogonial pair.   This four-part chromosome

(figs. 156a, 156b), after condensing, enters the metaphase plate in this extended condition.

Figure 156c is typical of the so-called cross form. The four parts are visible. The proximal knobs (x, x) appear similar to those of figure 156b. The arms of the cross extending along the axis of the primary split (I, I) may be bent about until their ends are almost in contact, as in no. 6, figure 150b, or even in contact, giving a ring-shaped chromosome, as in nos. 4, 6, and 9, figure 155.

The condition shown in figure 156d may be produced from that of figure 156c by a lengthening of the arms along the axis of the primary split and by so bringing their distal ends into contact that the whole chromosome forms a ring. With the fusion of the distal ends to form the ring, the components in that region begin to separate along the *primary* plane of division. At the proximal part of the chromosome, where this stage has been reached, the primary split (I, I) prevails; in the middle part, the secondary split (II, II) prevails; and at the distal end, the primary split again prevails, giving two knobs slightly smaller than the proximal knobs (x, x). By decreasing the extent of the secondary split (thus lessening the diameter of the ring) and increasing the primary split (thus increasing the length of both proximal-knob [x, x] and distal-knob ends), we get as a result the conditions of figures 156e, 155 no. 10, and 152a. Starting again with the ring-like stage, by increasing the length of the distal-knob ends and then bringing these knobs into contact again at the extreme distal ends (thus forming a second ring whose plane is perpendicular to that of the first ring), and by then decreasing the length of the proximal-knob ends (x, x) and the extent of the first ring, we obtain a condition like that of figure 156f. I do not say that a continuous change takes place along the axes of the 'splits' so as to produce all these different forms in one chromosome, but rather that as the conjugated chromosome—split in two longitudinal planes, the primary and secondary (I, I and II, II), at right angles to each other—comes out of the synapsis period, its four parts present one or the other of these conditions, separating most along the primary or second-

ary plane according as the one or the other happens to predominate. During these changes of form the chromosomes are undergoing rapid condensation (fig. 155). As they reach the metaphase stage, we at first get conditions such as are seen in figures 157 and 158.

Figure 157 is a nearly polar view of an early metaphase. The no. 11 chromosome is similar to 156d; no. 10, to figure 156c, except that it must have had very much longer arms than the latter; no. 9, to no. 9 of figure 155a; and nos. 6, 7, and 8, to nos. 7 and 8 of figure 155 and to 156c. Whereas no. 6 in figure 155 is a ring, it apparently is not so in figure 157; but in Syrbula admirabilis it has been shown (Robertson, '08) that chromosomes which usually form rings do not always do so. No. 5 is a ring similar to no. 5 in figure 155, while nos. 1, 2, and 3 are rods similar to the corresponding numbers in figures 155 and to figures 156 a and 156 b.

Figure 158 is a lateral view with all chromosomes shown. No. 11 is a ring in side view; nos. 1 and 2 are rod-like; no. 4, a cross approaching a rod; and nos. 3 and 8, rings. All others are crosses. The sex chromosome is a rod. In the second spermatocytes (figs. 159, 160) there are two sorts of cells, depending upon the presence or absence of the sex chromosome. The size relations and rod-like character of the chromosomes can readily be made out here.

I have given a detailed description of Syrbula to prepare for a better understanding of the chromosomes in

2. *Chorthippus* (*Stenobothrus*) *curtipennis Scudd.* Chorthippus is unique among Acrididae in having apparently seventeen chromosomes. Three pairs of the chromosomes are V-shaped, each with arms of unequal length. This suggests possible compounding in chromosomes. These V's have the apices turned toward the center of the metaphase plate. At the apex is a thin place, seemingly a point of union, over which the deeply staining material is not continuous. If we assume that each of the six unusual V's embraces two chromosomes, the number seventeen is increased to twenty-three. If this assumption be correct, the chromosomes may be numbered according to

size as in Syrbula, with which the size relations are almost identical. For in Chorthippus there are two large 11's (designated as 12's in Robertson, '08), each linked with a no. 7; two 10's (11's in '08), each linked with a no. 8; and a sex chromosome, $10x$, as in Syrbula. Further, the two large 9's are linked each with a no. 5. The 6's, 4's, 3's, 2's, and 1's are separate, as in Syrbula. The gap in size between 8 and 9 is possibly larger, between 3 and 4 not so great, and between the 1's and 2's a little greater than in Syrbula; between 2 and 3 it is wide, as in Syrbula (figs. 184–187). In general, then, the size relations of chromosomes in Chorthippus are very similar to those in Syrbula.

As in Syrbula (fig. 149), conjugating chromosomes come out of the diffuse stage in parasynapsis (figs. 163, 164). In each of these cells all conjugated chromosomes (eight) and the sex chromosome are accounted for and drawn. In figure 163 nos. 2 and 4 have been drawn as though moved out radially from their positions; the real position of $10x$ is indicated by a plus sign $(+)$. In figure 164 chromosomes 1, 2, 4, and $10x$ have likewise been transferred outward. I wish to call attention to the three long chromosomes (7–11, 8–10, 5–9) in these two cells. All of the chromosomes show a longitudinal split, and in parts, or all, of each, a second such split at right angles to the first is evident (figs. 163, 164). In figure 163 an accidental (?) interlocking of the two largest chromosomes (7–11, 8–10) seems to have taken place. Both show the primary split farther advanced than the secondary. In 7–11 the halves gap apart in the middle region (x''', x'''') and include between them one of the halves (x') of 8–10, which is gaping apart similarly in its middle region (x', x''). The two chromosomes are interlocked like the links of a chain. This phenomenon may be of importance in the question of the continuity of the chromatin thread during parasynapsis.

The no. 4 chromosome in Chorthippus, as in Syrbula, is much condensed and deeply stained. Frequently this chromosome, or the end of one exconjugating limb of it (x in fig. 168), lies near one end of the sex chromosome (figs. 164, 165, 168, 170, 173, 174, 178b, 179, 180). In this respect it behaves similar to Mc-

Clung's chromosome in Hesperotettix and Mermiria, which likewise was associated with the sex chromosome, but more permanently than in Chorthippus.

The long chromosome (7–11) in figure 165 is clearly split into two strands and optical cross-sections at favorable points (a and b) show that each of the halves is again split. In the middle at the points x, x, the halves gape apart. These points correspond to what would be the proximal points of chromosomes 7 and 11, were they going through this pairing process separately, as they do in Syrbula (fig. 149).

In figures 166, 167a and 167b are chromosomes in the same stage as figure 164, which show clearly the four longitudinal parts. The black points at the ends are probably the polar granules. In figure 168 is a still later stage where the chromosomes are more condensed, but still show their two longitudinal splits and the resulting four strands. At any point along the chromosomes the strands are usually more closely apposed in one direction than in the other, according as the primary or the secondary split is farther advanced. The letters "x, x" mark the points where the attraction fibers were destined to spring forth, for at these points there is a constriction in the long chromosomes (7–11, 8–10, 5–9). In figure 164 nos. 10x and 4 have been drawn outside the nucleus for convenience. Figure 173 is of a still later stage. Chromosomes nos. 3, 6, and 7–11 (173a) have been drawn outside the nucleus (173b) for clearness. The sex chromosome, as usual, lies near no. 4.

In the next older stage (fig. 174) the chromosomes have become more condensed. No. 4 takes on its usual shape, a modified cross. Nos. 1, 2, and 3 are rods, which have already opened out by rotation so that the primary split is represented solely by the region of contact where the distal ends of the halves resulting from this split still meet. The compound nature of nos. 7–11 is evident. The proximal points (x, x) are marked by constrictions. The smaller of the two chromosomes, no. 7, forms an almost complete ring in the plane of the drawing paper. The larger (no. 11) part forms a ring similar to that of figure 156d. The ring perpendicular to the plane of the paper is the link in

the middle formed by the junction of the proximal knobs of
7 and 11. In 5–9 the middle, perpendicular ring is much in-
creased in extent at the expense of the horizontal[4] portion of
no. 9, which is a cross, and of no. 5, the halves of which are
merely in contact. But in 8–10 the separation is such that
we have three rings. The middle, perpendicular ring has con-
strictions (x, x) near the middle of each part, the points of junc-
tion between 8 and 10. The plane of the terminal rings is at
right angles to that of the middle ring. They may be referred
to as horizontal rings.[4] Their distal parts have split so as to
form crosses, the planes of which are parallel to that of the
middle ring. No. 6 clearly has the form of a cross. In all
of the autosomes the four longitudinal parts are evident in places.
I believe that they have originated from conditions like those
shown in figures 164, etc., where each of the eight ordinary
chromosomes consists of four longitudinal strands lying close
together.

Still later prophases of the 7–11 and 8–10 chromosomes are
shown in figures 175 and 176. In figures 176 and 177 especially
the points of junction (x, x) between chromosomes 7 and 11 is
very evident on each of the four strands of the middle ring.
Here, too, the four longitudinal parts of the chromosome may
be very readily made out. An unusual occurrence is illustrated
in figure 177. One of the two compound chromosomes in con-
jugating has evidently at some time, probably during parasynap-
sis, enclosed the other. Here, at the end of the period, they
are still in the same relation to each other and neither seems to
have been affected by it.

Figures 178a and 178b are of a late prophase showing all nine
chromosomes. The constrictions at x, x indicate the com-
pound character of the three largest pairs, which is also shown,
even more distinctly, in the early metaphase of figure 179.

A careful comparison of this figure (179) of Chorthippus
with the corresponding figure (157) in Syrbula is very instruc-

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[4] I use the term 'perpendicular ring' to show that this portion of the chromo-
some, when the latter takes its position on the spindle at metaphase, will be
perpendicular to the plane of the metaphase plate, and 'horizontal ring' to desig-
nate the portions parallel with this plane.

tive. Keeping in mind that in figure 157 the chromosomes are viewed in a direction (nearly parallel with the spindle axis) almost at right angles to that in which they are seen in 179 (nearly perpendicular to the spindle axis), it will not be difficult to imagine the results of bringing together in twos, by means of attachments at x, x, the separate chromosomes 7 and 11, 8 and 10, 5 and 9 of figure 157. The results would be chromosomes like those in figure 179. It is perhaps necessary to explain further the condition in some chromosomes of figure 179. No. 9 is a ring seen edgewise, with the distal region turned toward the observer; no. 5 is seen edgewise, but instead of forming a closed ring (fig. 157) its distal ends are free. The uncombined chromosomes (1, 3, 4, 6) are sufficiently alike to render their identification in the two figures reasonably certain. It will be observed, however, that there is no chromosome in figure 179 corresponding to the no. 2 of figure 157. This is probably due to the removal of that chromosome in an adjacent section, for there is present no other very small chromosome comparable in size with no. 1.

Figure 180 shows clearly the compound nature of chromosomes 5–9 and 7–11 in the fact that at the junctions (x, x) the deeply staining chromatin material is interrupted.

Figure 181, a late metaphase, shows the compound chromosomes, 5–9 and 7–11, in final stages of division. They give the familiar E-shaped picture, so often seen in Chorthippus and other species having V-shaped chromosomes, in which one arm of each V is longer than the other.

Figures 182a, 182b, 182c are lateral views of successive stages of a compound chromosome during the metaphase of the first spermatocyte division. The horizontal arms of the no. 7 part continue to get shorter. The perpendicular arms of 11, seen in 182a, have disappeared, or do not occur in 182b and 182c. Probably they never existed here; but there is a decrease in the proportionate size of the horizontal part of 11. The vertical ring drawn in figure 183 is probably an 8–10 compound, for the arms of each V are more nearly equal in length (compare with figs. 184, 185). The 8's are in contact by their distal ends only,

whereas the 10's are still broadly in contact along their horizontal arms, which thus still retain the familiar cross-shaped tetrad appearance of late metaphases, such as we find in species like Syrbula, where only rod chromosomes are found.

The second spermatocytes in Chorthippus are illustrated by figures 184 to 186, in one of which (fig. 185) the sex chromosome is wanting. An anaphase stage, showing the sex chromosome, is given in figure 187. In each of these four cells are three large V-shaped chromosomes. If each arm of a V be counted as a single rod-chromosome, there are readily seen to be 11 or, with the sex-chromosome, 12 chromosomes, which may be arranged in a graded series according to size, as in the case of the 11 (or 12) rod-chromosomes of Syrbula (figs. 159, 160). Believing that this is the true condition of the chromosomes, I have given them here corresponding numbers. It will be seen that no. 11 is attached to 7, 10 to 8, 9 to 5. The points of attachment are constricted and show clear spaces, as usual, where the two chromosomes have become united. The size relations of V's, and of all the other chromosomes, are the same as in first spermatocytes and spermatogonia.

3. Chorthippus biguttulus Linn. For sake of comparison with C. curtipennis I have copied two of Gerard's ('09, p. 582) figures of C. (s.) biguttullus, and have attempted to number the **V**-chromosomes (my figs. 188, 189) like those in curtipennis. From the lengths of the limbs of the three **V**'s shown here, one would conclude that probably the same chromosomes are concerned in forming the **V**-compounds in biguttullus, a European species, as in curtipennis.

4. Résumé on Chorthippus and Syrbula. (1) The study of tettigidean chromosomes has shown that the same numbers and, within certain limits, the same size relations are found among all the genera of a family. Syrbula and Chorthippus are closely related genera of the acrididaean subfamily Truxalinae. They differ *apparently* in the number of chromosomes. Both have one (♂'s) or two (♀'s) sex chromosomes. Syrbula has eleven pairs of rod-shaped autosomes; Chorthippus has three pairs of **V**-shaped and five pairs of rod-shaped autosomes.

(2) In Syrbula these rod-shaped autosomes and the sex chromosome may each be recognized in a group by their relative lengths. Almost the same size relations may be seen in the sex chromosome, the five pairs of rods and the six pairs of limbs of the three pairs of **V**'s of Chorthippus.

(3) A constriction, a clear, non-staining bridge, and an attraction fiber occur at the apex of each of the **V**'s in spermatogonia. These conditions may be traced in spireme stages, the prophases, metaphases, and anaphases of first spermatocytes, in second spermatocytes, and in spermatids. Such constrictions divide the members of each pair of **V**'s into two pairs of rods, equivalent in length in each case to the 5's and 9's, the 7's and 11's, and the 8's and 10's of Syrbula.

(4) The limbs of the paired **V**'s in late synapsis stages, prophases, and metaphases of the first spermatocytes behave like the 5's, 7's, 8's, 9's, 10's, and 11's of Syrbula, in that they form, during these stages, rod-shaped, cross-shaped, horseshoe-shaped, and ring-shaped tetrads, depending, as in the rods of Syrbula, upon the lengths of the pair of limbs in each case. They agree also in the characteristic appearance that each such tetrad gives in the late metaphase and in anaphases. The diads resulting from these behave similarly in the second spermatocytes of both genera.

(5) In conclusion, therefore, I believe that the three pairs of **V**-autosomes of Chorthippus are the equivalent of the six pairs of rod-autosomes of Syrbula,—the 5's, 7's, 8's, 9's, 10's, and 11's, —and that the remaining five pairs of rod-autosomes and the sex chromosome of Chortippus are likewise the equivalent of the 1's, 2's, 3's, 4's, 6's, and sex chromosome of Syrbula. If this be so, then we may consider each of these three pairs of **V**'s to be a compound chromosome, and that, therefore, the Truxalinae have the number twenty-three, characteristic of Acrididae.

3. Oedipodinae (Acrididae)

The third subfamily of the Acrididae is here represented by Chortophaga viridifasciata and Dissosteira carolina (figs. 190,

191, after Davis, '08, p. 72). As in Syrbula, there are twenty-three rod-shaped chromosomes. Among these are the usual three large pairs. The sex chromosome does not rank as 10, but as 8 (Dissosteira) or 9 (Chortophaga). As in Syrbula, there are three very small pairs in Chortophaga; in Dissosteira two of the three are exceedingly small, one (no. 3) being, however, considerably larger, as in Chorthippus, though still worthy of being grouped among the three small pairs. The 4's, 5's, 6's, 7's, and in Dissosteira the 8's form a continuously graded series of sizes similar to the conditions in Syrbula.

4. Acridiinae (Acrididae)

In this, the fourth subfamily of Acrididae (fig. 192), we again have twenty-three chromosomes. The size relations differ considerably from those of the oedipodines and truxalines, though there are agreements. There are, as usual, two very large pairs, but the third largest (9's) is smaller than in these subfamilies. The sex chromosome ranks 10. There is a considerable gap between $10x$ and the 9's, but the 9's, 8's, 7's, 6's, and 5's form a graded series with less differences in size than in the truxalines and oedipodines. The 4's are much smaller than usual, though still noticeably larger than the 3's. We can therefore say that for these three subfamilies of the Acrididae the number of chromosomes is twenty-three ($\male$).

5. Locustidae and Gryllidae

V-shaped chromosomes are very common among the Locustidae and Gryllidae. The number of chromosomes in Locustidae seems frequently to be thirty-one or thirty-three. In cases where a smaller number occurs, some of the chromosomes are V-shaped, as in Chorthippus. By counting each of these as two chromosomes, the total number becomes thirty-one. This may be seen by comparing figure 193 of Decticus verrucosus (after Buchner, '09, fig. 82) with figure 202 of Steiroxys trilineata (after Davis, '08). If we assume that the two large V's in the latter are twofold, the number in reality is thirty-one.

Baumgartner ('03) found in the field cricket (Gryllidae) twenty-one chromosomes, whereas in the house cricket (Gryllis domesticus) there were twenty-nine. His figures of the field cricket show similar, though small, V-shaped chromosomes, whose apices are turned inward (figs. 203, 204). By counting each of these V's as two chromosomes, the number here also is twenty-nine.

That my suspicion is well-founded in regard to V-chromosomes, has just been shown by the work of one of my students, Miss Woolsey, upon three species of Jamaicana (Locustidae). Figures 194–201 have been taken from her paper (Woolsey, '15). One species of this genus always has thirty-five (σ's) rod-shaped chromosomes (fig. 194, spermatogonium). A second species may have thirty-five (σ's) or thirty-four (σ's) chromosomes. When the number is thirty-four, one large V-shaped chromosome is always present (spermatogonium, fig. 196, no. 14–16). A third species shows either thirty-five rod-shaped chromosomes or a group of thirty-three in which there are always two equal-sized large V-shaped elements (nos. 14–16, fig. 200, spermatogonium). In prophases and metaphases of the first spermatocyte, individuals of the 35-rod type present seventeen rod-shaped tetrads similar to those of Tettigidae, in addition to a very large sex chromosome (18x, fig. 195). In the 34-chromosome individuals, where one V is present, the first maturation division shows fifteen rod-shaped tetrads plus the sex chromosome plus one long V-shaped chromosome (octad), which evidently represents the V-chromosome of the spermatogonium joined to two of the rod-shaped spermatogonial chromosomes (14, 14–16, 16, figs. 197–199). Careful measurements of the length of the limbs of the V in spermatogonia and first spermatocytes and of the chromosome attached to the end of each limb of the V in first spermatocytes show each limb to be of the same size as the chromosome attached to it (figs. 198, 199). Each attached chromosome (14, 16) is therefore probably homologous with the limb of the V-type multiple chromosome to which it is joined.

In figure 198 it is noticeable that one limb of the V is longer than the other. In the pairing which takes place during the first maturation, it is to be expected that the long limb will pair

with a similar long-rod chromosome and the short limb with a similar short-rod chromosome. There would then result in first maturation divisions a long **V** with unequal limbs, exactly what is found (figs. 198, 199). At the end of this division the limbs of the original spermatogonial **V** separate from their rod mates and the **V**, passing to one of the second spermatocytes, has the same size as a spermatogonial **V**.

In two-**V** type individuals, first spermatocytes show the sex chromosome, fifteen rod-shaped tetrads, and an elongated ring (fig. 201). This ring evidently results during the exconjugation process of the pair of **V**-shaped chromosomes. The longer (16, 16) and shorter (14, 14) sides of this ring probably contain the same elements as are present in the longer and shorter limbs of the first spermatocyte **V**'s of figures 197 to 199. I have examined carefully every cell drawn by Woolsey and am reasonably certain that in Jamaicana we have cases of linkage of non-homologous rod chromosomes to form **V**'s, etc., which are entirely similar in their behavior to the **V**'s of Chorthippus. Jamaicana is important because it shows the transition from rod-shaped to **V**-shaped chromosomes and a corresponding reduction in the number of chromosomes, not only within the genus but within each species. It shows in the individuals studied that two chromosome pairs may exist as four rods, or as two rods, and one **V** or as two **V**'s. At the same time there are present in each case thirty additional rod-shaped chromosomes and the sex chromosome, making always thirty-five rod chromosomes or their equivalent.

DISCUSSION

1. **V**-shaped chromosomes

1. General. I undertook an examination of the **V**-chromosomes of Chorthippus in an attempt to find if such **V**'s were not in some way concerned in bringing about the smaller number, seventeen, which all species of this genus seem to have. The number characteristic of the subfamily to which this genus belongs, as well as of the two other subfamilies closely related

to it—the Oedipodinae and Acridiinae—had up to this time been thought to be twenty-three. As a result of the comparisons of chromosomes in this genus with those of the related genus Syrbula and with those of some of the Oedipodinae and Acridiinae, and in view of what I have found to be true of chromosome numbers and sizes among the genera of the Tettigidae, and especially in the light of the relation known to exist between rod- and V-shaped chromosomes in three species of Jamaicana, I am convinced that the smaller number in Chorthippus is due to the presence of V's. I am reasonably certain that these V's, such as we find in Chorthippus and Jamaicana and possibly in other locustids and gryllids, have resulted from the linkage proximally of two non-homologous chromosomes, and that the non-chromatic bridge at the apex of these V's,—sometimes accompanied by a constriction,—from which the spindle fiber springs, is the point at which this linkage has taken place. With these conclusions in mind, I turn to a consideration of V-shaped and segmented chromosomes described by others, and the possible relation of such chromosomes to some of the problems of cytology and genetics.

V-shaped chromosomes are common in both plants and animals. They are referred to in cytological literature as 'equal armed' and 'unequal armed' (caudés) V's or U's, or as being 'hook-shaped' or 'J-shaped' (Grégoire, '05, '10). The spindle fiber springs from the apex of the angle formed by the arms and is referred to as coming from the median, a near-median (intermediate), or a subterminal region. Besides these V's, the only forms of chromosomes we have, except in first-maturation divisions, are straight rods and, possibly, spheres. In the straight-rod type the spindle fiber usually springs from the end and is spoken of as terminal. However, in Copepoda, where the rods show transverse segmentation, the attachment of the fiber may be median or sub-median. But in anaphases such rods appear as V's and therefore may be so classed. For convenience, I use the term V-chromosome in a general sense to include all of these two-armed chromosomes, viz., the V's, U's, and J's, and transversely segmented rods. However, I do not consider

as **V**-chromosomes the '**V** *simples*' of Grégoire ('10, fig. 65a), which occur in both anaphase and telophase of the first and in the metaphase of the second maturation division (Robertson, '08, figs. 37–39; Davis, '08, figs. 65, 93; the present paper fig. 160, nos. 7, 10, and 10*x*). These diads are in reality rod-shaped chromosomes, equivalent to spermatogonial rods split longitudinally, whose halves have a tendency to gape at the distal end. The same appearance is frequently seen in spermatogonial and somatic metaphases in Syrbula, where we are certain of having only rod-shaped chromosomes from which such so-called **V**'s may be derived. Frequently in illustrations of the anaphase of first spermatocytes, or the metaphase of second spermatocytes, the outlines of the halves of these chromosomes are overlooked, especially at the apex, so that the chromosome is made to appear as a true **V** or **U** (Hartman, '13, figs. 33–47). I believe that the failure of authors—Rückert and his followers, who worked on copepods, and Belajeff ('98) and Ishikawa ('97) on plants—properly to distinguish between the '**V** simples,' derived from the rod-type tetrad in anaphases of the first and in metaphases of second maturation divisions, and the true **V**-shaped type of chromosome during these stages, has led to confusion in causing an interpretation of the second maturation division as exhibiting post reduction. Furthermore, I do not include among the true **V**'s the '**V**-tetrads' of insects (nos. 7, 8, and 10 in fig. 157; McClung, '14, figs. 92, 93), whose **V** form I consider to have resulted from the moving apart, along the axis of the secondary split (II, fig. 156c), of the distal ends of the parts of a tetrad which originally consisted of four strands lying side by side (Robertson, '08, fig. 29, *e* and *h*).

In going over the literature on these chromosomes, it has seemed to me that possibly there may be two types of **V**'s. In the first the chromosome may be considered a bent rod having no achromatic bridge nor marked constriction at the apex of the **V** or **U**. Such chromosomes, if short, would tend to be rods, straight or slightly curved; if long, they would be **U**'s. The **U**-shape would be especially brought out in anaphases of division figures. Chromosomes of this type may be seen in

Ascaris, Tomopteris (Schreiner, '06a), in amphibia and, possibly, among the chromosomes of an unidentified acridid described by McClung ('14, figs. 59–62). McClung suggests that "V's may have been derived from straight rods through an altered transverse segmentation of a continuous spireme thread, which would result in chromosomes with fiber attached at a subterminal or median position, instead of a terminal." Such V's might be grouped with the bent-rod type.

In the second group the V's bear at the apex unmistakable achromatic bridges of clear non-staining material. At this point there is also a constriction. Such chromosomes I believe to have been derived in many cases from the linkage proximally of two non-homologous rods (nos. 8 and 10 in Chorthippus, nos. 14 and 16 in Jamaicana unicolor). In this type may also be placed those chromosomes which are loosely linked together, possibly in the stage of forming V's (Browne ['10, '13], in Notonecta insulata; Woolsey ['15, figs. 5–8, A], in Jamaicana flava).

As to the validity of the former group, I am unable to say. I merely suggest that such may exist (Agar, '12, fig. 9). By far the larger number of the V's and U's which have been figured have been represented as such, exhibiting neither constrictions nor transverse clear regions and would therefore be grouped with this type. But certainly not all such described V's may be so grouped.

I am certain of the occurrence of the second type of V's. I also believe that this type will be found to be much more frequent than one might suppose, for in the case of the genus Chorthippus (Stenobothrus) alone, de Sinéty ('01), Davis ('08), Gerard ('09), and Meek ('11, '12a) have overlooked the transverse segmentation which I describe here. I should expect to find this linkage type of V in those genera which show a smaller number of chromosomes than is characteristic of the family to which they belong, and at the same time a relatively larger number of V-shaped chromosomes—conditions which are found in comparing Chorthippus with other genera of the Truxalinae.

2a. **V**-*shaped chromosomes in plant-cell mitoses, not including prophase and metaphase of the first maturation division.* True V-shaped chromosomes have been described in Lilium, Allium, Trillium, Helleborus and Podophyllum. In Lilium they have been shown by Grégoire ('99, '10), Strasburger ('00), and Mottier ('03, figs. 6, 8, 16, 41). Only in figures 16 and 41 does Mottier present any indication of transverse segmentation. Grégoire's ('99) figures show V's, but, with the exception of 19*b*, *c*, and d and one or two chromsomes in figures 22 and 23, it is impossible to distinguish between the true V's and his V-simples. Mottier describes these chromosomes as straight rods, bent rods, and U's. His figures indicate that for the genus Lilium probably a large number of rods is characteristic. Bonnevie ('11, figs. 12, 13) finds in Allium both V- and rod-shaped chromosomes (figs. 55–60). Like Grégoire, she has failed to distinguish between true V's and V-*simples*. The former show a split along each arm of the V in the anaphase. Mottier found similarly shaped chromosomes in Podophyllum (figs. 22, 23), in Tradescantia (fig. 32), and in Helleborus (see Grégoire, '09, fig. 19). As to whether any of the V's in the genera described would on re-examination show the segmentation at the apices, I cannot tell, except in the case of Podophyllum, where in root-tips cells I have found segmentation similar to that shown in Chorthippus.

In Trillium, Grégoire et Wygaerts ('04) have observed three cases of segmentation, of which they say,

Nous observons très souvent dans les chromosomes-filles une ou plusieurs fentes transversales, très précises, comme tailleés au couteau, Fig. 20. Elles se correspondent parfaitement d'un segment-sœur à l'autre, Fig. 20. Il est certain qu'il ne s'agit pas là d'une fente complète; le chromosome en effet ne se disloque jamais; mais nous n'entrevoyous pas d'explication de ce phénomène, que nous ne faisons que signaler.

This transverse segmentation corresponds with what I have found in Chorthippus. Since at least five of the seven pairs of chromosomes in this genus are V's, as figured by Atkinson ('99) and by Grégoire ('09); since many of these chromosomes have unequal arms, almost identical to my no. 7–11's or 8–10's in

figures 162, 186, and 187; and since the number is small compared with that of other genera of the lily family (which possess twenty-four), I believe that Trillium may possibly present a case of compound chromosome formation similar to that of Chorthippus.

2b. V-shaped chromosomes in somatic, spermatogonial, and second spermatocyte divisions and in anaphases of first spermatocytes. Beginning, among animals, with nematodes, we find Ascaris megalocephala to have in one variety, four; in the other two chromosomes, which appear to be of the **U** type (Boveri, '88, figs. 44b, 60, 61). The spindle fibers are attached not at any one point but along the whole mid-region. The only indication of constriction which has been figured is that of the first maturation chromosome, shown by Tretjakoff ('04, fig. 7).

In annulates the Schreiners ('06a, figs. 2, 6, 8–12) describe **V**-shaped chromosomes of the **U** variety. There is a long narrow constriction in the middle but no indication of segmentation such as exists in Chorthippus or Trillium. In Nereis Bonnevie ('06, pp. 62, 63) has demonstrated **V**-shaped, **J**-shaped, and rod-shaped chromosomes in somatic cells.

After an examination of the cell studies on Copepoda by Rückert ('93), Haecker ('95, '02, '11), Lerat ('05), Krimmel ('10), Braun ('09), and Matscheck ('10), I have come to think that possibly a good deal of the confusion in respect to variation in chromosome numbers, heterotypical mitoses, etc., has been due to the presence of compound chromosomes of the form, either of **V**'s, bent rods or straight rods, possessing a segmentation similar to that which I find in Chorthippus. Lerat ('05, figs. 25–32, 46–50) has shown that three types of chromosomes exist in the copepods as in other groups of animals and in plants; namely, straight rods with terminal attachment of fibers, bent rods or **V**'s with subterminal attachment of fibers, giving **J**'s or hooks, and **V**'s with either median, or submedian attachment of fibers. The occurrence of these three types of chromosomes, especially the straight-rod type, has been overlooked by the other investigators of the group, though Matscheck in his figure 8 gives one rod chromosome occurring along with

three **V**'s, and Haecker himself has also shown **V**'s ('11, figs. 22, 30, 65).

But we do not always find **V**'s in the copepods as described by Haecker, Matscheck and others. This is possibly due to the fact that the limbs of what might be regarded as straightened-out **V**'s are usually so short that they have a tendency to lie in one axis instead of at an angle to each other, and also that in some of these species there may be few compound chromosomes. The **V** structure, if present, appears in anaphases, whether the limbs are short or long. Lerat's cells were evidently in the right stage to show **V**'s.

The best evidence of the compound nature of the chromosomes in Copepoda is the presence of a constriction, leaving an archoplasmic bridge, median or submedian in position, which gives to the chromosome the appearance of being transversely segmented. Lerat denies the presence of the constriction or break, but admits the presence of a non-staining region. His figures, however, show constrictions at the apices of the **V**'s. This segmentation has been figured by Rückert ('93), Schiller ('09, figs. 7–19), Haecker ('95, '02), Krimmel ('10) and Matscheck ('10). Rückert (see Lerat, '05, p. 181) was the first to describe the phenomenon of segmentation in chromosomes, though probably he did not understand its meaning. Haecker ('11, p. 45) describes this phenomenon when he says, "Seltener ist eine durch helle Querkerben hervorgerufene *wirkliche* Segmentierung wahrzunehmen, so z. B. bei den 'bivalenten' Chromosomen der Kopepoden (fig. 14b) und offenbar auch bei den auffallend langen 'Sammelchromosomen' von Ascaris." He has, however, in my opinion, misinterpreted this bridge as representing a stage in the reduction process, in accordance with his metasyndesis theory, which postulates that at some time during the life cycle of the animal after fertilization of the egg and before the first maturation division takes place, there occurs an end-to-end synapsis of like chromosomes, resulting finally in a fusion during the growth stages of the first maturation cell. It will be seen that, according to Haecker, the transverse segmentation of chromosomes in the Copepoda does not persist as a permanent

region of the chromosome, but must appear and disappear with the life cycle of the animal. He looks upon it as the point of conjugation of two *homologous* chromosomes, the halves of the rod (limbs of the **V**) being homologous chromosomes and, of course, equal in length. That this is not the case, is shown by the fact that the limbs of the V are frequently of unequal length (Lerat, '05), as in Chorthippus and in so many species with **V**-shaped chromosomes.

The variation in chromosome numbers which Krimmel ('10) found in somatic mitosis of Diaptomus—between twenty-eight and fourteen (reduced number)—Haecker ('11, p. 113) explains as follows: "Man wird die intermediären Zahlen auf einen unvollständigen Zerfall bivalenter Elemente, also auf eine Mischung bivalenter and univalenter Elemente zurückführen dürfen." Haecker is probably right in ascribing numerical variation to breaks at points of segmentation, though I believe he is wrong in thinking that the 'univalent' chromosomes making up these 'bivalents' are to be considered as members of homologous chromosome pairs.

Schiller ('09, figs. 7–9) describes tetrads in somatic cells of Copepoda. He probably saw some of these compound chromosomes with transverse segmentation in which the longitudinal split is visible, as in Trillium (Grégoire et Wygaerts, '04, fig. 20). In such a chromosome one might see four parts and thus mistake it for a tetrad (compare fig. 134).

Among the insects **V**-chromosomes have been found in the phasmids by de Sinéty ('01); in five acrididaean genera; in Stenobothrus by de Sinéty ('01), Davis ('08), Gerard ('09), Meek (10', '11, '12) and myself, in Hippiscus, Mermiria, Hesperottix, and Chortophaga by McClung ('05, '14); in the Locustidae by Woolsey ('15), Stevens ('12), McClung ('05), Buchner ('09), Vejdovsky ('11–12), Davis ('08); in the Gryllidae by Baumgartner ('04) and Payne ('12); and in Coleoptera (Coptocycla) by Nowlin ('06).

In the phasmid Leptynia attennuata Pant., de Sinéty shows in spermatogonia (figs. 73, 75) three **V**'s among thirty-six chromosomes. In Stenobothrus three pairs of **V**'s, such as I have found, have been described by all authors who have investigated this

genus (de Sinéty, Gerard, Davis, Meek). With the exception of Davis ('08, figs. 17, 21), none of these authors have seen the archoplasmic bridge or constriction at the apex of the V's. Though Davis's figures (17–21) show it, it is not mentioned in the text. The V's in every case have arms more or less unequal in length.

McClung ('05, figs. 1, 7, 8, 9; '15, fig. 84) has described a single V-shaped chromosome in three species of Hesperotettix (♂) which is made up by linkage proximally of an autosome with the sex chromosome. The sex-chromosome portion may be recognized by the smoothness of its surface. In Mermiria also he has described a V-chromosome; this differs from that of Hesperotettix in that the sex chromosome here seems to be attached to the distal end of one of the two autosomes entering into the V. McClung believes that in this multiple chromosome of the first spermatocyte division two tetrads, as well as the sex chromosome, are concerned, and that in this division whole tetrads separate from each other. It seems to me that, instead of interpreting the multiple body in this way, we may say that Mermiria has a pair of autosomal V's, to the end of one of which is appended the sex chromosome. The presence of the sex chromosome in the V's of both genera is evidence of the compound nature of these V's, since one limb of the V may be identified by its smooth surface as an individual, specific chromosome (viz., the sex chromosome), while the other limb is shown by its roughness to be similar to the autosomes. Also the number of other autosomes present shows that it must be one of them.

The V's of Chortophaga, which McClung (fig. 94) interprets as the result of the precocious synapsis of homologous chromosomes in the spermatogonia, I am inclined to think are not such; they are, instead, V's consisting of non-homologous chromosomes, similar to those of Chorthippus, in which, however, the fusion is more like that of Notonecta (Browne, '13) or Jamaicana (Woolsey, '15) than of Chorthippus. The non-homologous nature of the limbs of the V's in all these cases may be inferred from the fact that they are always of unequal length.

In the Diptera Stevens ('08) describes **V**'s in nine genera. She has not distinguished, however, between the true **V** and the 'V-simples' of Grégoire. No points of constriction or segmentation at the apices are in evidence except in Drosophila ampelophila (figs. 57–82), in which there are two pairs of **V**'s, a pair of *M*-chromosomes, and a pair of unequal sex chromosomes. Metz ('14) has found in eight species of the genus unmistakable evidence of the compound nature of these **V**'s, since in some species one pair of **V**'s may be replaced by two pairs of rods, and in other species both pairs of **V**'s may be replaced by four pairs of rods.

Among vertebrates the Dipnoi (Lepidosiren) and the Amphibia afford striking examples of **V**-shaped chromosomes. In Amphibia from ten to eleven of the twelve pairs of chromosomes are **V**-shaped, and only one or two pairs rod-shaped; e.g., Flemming ('87, figs. 41, 42, 43a, 44) and Drüner ('94, '95) in Salamandra; Eisen ('03), Janssens et Dumez ('03), and Janssens ('05, figs. 69–71) in Batrachoseps; Carnoy et Lebrun ('99, figs. 103, 104, 118) in Triton; Lebrun ('02, figs. 33–35, 41, 42) in oögenesis of Diemyctilus; Montgomery ('03) in Desmognathus and Plethodon, and Muckermann ('12) in urodeles.

The arms of the **V**'s in Amphibia are seldom of equal length (Janssens, '02), as is clearly shown by Muckermann in somatic mitoses, where (his figs. 1–4) ten pairs are either **V**'s or **J**'s and only two pairs are rods. Three pairs of the **V**'s have sharp-angled apices, and some appear constricted at this point, as was also shown by Eisen ('00, figs. 112, 120h, 120k). The constriction, accompanied by a clear non-staining bridge, was shown by Montgomery ('03) in the chromosomes of the first spermatocyte, which were in synapsis.

That transverse segmentation occurs in Amphibia is clear from the works of Meves ('07) and Della Valle ('07), who describe in somatic cells from various tissues of Salamandra chromosomes thus segmented and showing at the same time the longitudinal split. According to these authors, and others who describe similar figures (Bonnevie, '08, '11; Popoff, '08; Haecker, '07), these are examples of heterotypical (tetrad-like) divisions

in somatic cells. They argue that if such conditions are found in somatic cells, where only longitudinal divisions occur, the similar heterotypical division of the first maturation may likewise be interpreted as longitudinal. The second is like it, therefore no reduction occurs. The question of heterotypical division we shall discuss later. Their observations, however, are evidence that we are dealing with at least some transversely segmented, and possibly compound, chromosomes in Amphibia.

In Lepidosiren Agar ('11, figs. 6, 7) found thirty-eight chromosomes. Of these, thirty-four are clearly either V's or hook-shaped, four are rod-shaped. He notes a transverse constriction across each of the thirty-four univalent chromosomes, as he calls them, and says ('11, '12) that it corresponds in the spermatocyte divisions with the apices of the V's of the somatic or spermatogonial mitoses. This is in exact agreement with my results on Chorthippus. He also shows ('12, p. 291) that for each chromosome this constriction always lies at a certain point, whether the limbs are equal or unequal. This is described for the five largest chromosomes. One pair always has limbs of nearly equal length in all mitoses, while the four pairs next in size are always hook- or J-shaped. This resembles Chorthippus, where there are always three pairs with arms of unequal length.

In mammals van Hoof ('12) figures in spermatogonia (figs. 01–2, 03–6, to 03–8) and anaphases of the first spermatocyte (plate III, figs. 026–48d; plate IV, figs. 027–50, 01–65, 01–66) rod-shaped, J-shaped, and V-shaped chromosomes. A few of his V's have sharp apices, where they show a constriction. I think that in man, according to the work of Wieman ('13), there is evidence of compound chromosomes in the transverse segmentation, which is clearly shown, and in the presence of many short V-shaped chromosomes. The great disagreement in the number of chromosomes reported for man may be due, in my opinion, to the extent of this compounding in chromosomes. Winniwarter ('12) reports forty-seven for a Caucasian, while Guyer ('10) and Montgomery give twenty-two ($\male$) and twenty-four ($\female$) for the negro. Wieman finds an intermediate num-

ber, about thirty-three to thirty-eight. Among these are a large number of **V**'s with transverse segmentation. He says: "The small chromosomes may be derived by a breaking up or 'diminution' of the larger ones. Likewise the difference between the somatic number and the spermatogonial number (as reported by Duesbug, Guyer, and others) may have a similar explanation."

From the preceding survey it may be seen that **V**-chromosomes, with either short or long arms, are of common occurrence in most families. We are led to suspect that transverse segmentation (not always shown) and constrictions, when they occur, together with non-terminal attachment of fibers may possibly be correlated in many cases with decrease in numbers brought about by a compounding of non-homologous chromosomes. We also are led to think that a **V**-chromosome may break at the apex to form two rods.

3. **V**-*Chromosomes in synapsis and reduction.* A general review of the literature upon these stages has been given, either completely or in part, by Grégoire ('05, '10,) Haecker ('07, '10), Davis ('08), Granata ('10), Wilson ('12), and others. It is necessary, however, to consider the literature which bears upon the **V**-chromosomes, since they are of such wide occurrence and possible importance. I shall try to show where I think others have failed to interpret correctly the behavior of these chromosomes, and wherein my work, together with the work of others on these and other chromosomes, furnishes evidence in favor of parasynapsis, but opposed to a complete fusion of the pairing threads, also in favor of the first division being reductional.

V-shaped chromosomes, since they are of such wide occurrence, have, I believe, played a considerable rôle in the debate over the synapsis stages, for much confusion has resulted from the failure of investigators to appreciate correctly the difference between chromosomes of the **V** or **J** and the straight-rod types during synapsis and following periods. The first stage in which misunderstanding has occurred is the so-called 'bouquet stage.' Janssens ('03, '05), the Schreiners ('06a, '06b, '08), and others have shown conclusively that, following the

parallel arrangement of the leptotene threads, the side-to-side pairing of homologous chromosomes begins at the free ends of the members of each pair; i.e., the ends which lie at the distal pole of the cell, and advances along the pair toward the opposite ends. In the case of **V**'s, pairing should begin at the distal ends of their limbs, and move toward the apices (Schreiners, '06a, figs. 20–26). The distal ends of the chromosomes lie in the distal part of the cell; i.e., that part which contains the interzonal body and is nearest to the plane of the last division (Davis, '08, p. 81). There would then result from **V**-pairs—of which according to Muckermann ('13) there are in urodeles about seven or eight—the familiar loops of the 'bouquet stage,' characteristic of so many species.

In those species where no **V**'s, but only straight rods, occur the loops, such as Janssens ('05, fig. 42) and the Schreiners ('06a, figs. 20f, 20h, 22, 25, 26) have shown in this stage, would not appear. I have found this to be true in the Tettigidae (my unpublished "Study II"), where this stage, from its resemblance to a sheaf of wheat, might appropriately be termed the 'sheaf' stage. Wilson ('12, p. 387) has not been able to find the 'bouquet-stage,' nor the polarization that goes with it, in any of the Hemiptera. Here the chromosomes are short, which may account for the lack of polarization. Davis ('08, figs. 31–34) describes for Dissosteira (a rod-chromosome genus) what he took to be the loops in the 'bouquet-stage;' but I think he has wrongly interpreted as 'loops' what I believe are the much bent, long rod-chromosomes ('10s and 11's of Syrbula) intimately paired side-to-side. The extreme length of the paired thread in such cases has caused it to bend and simulate the loops of Tomopteris or Batrachoseps. But if one look carefully at Davis's figures, it will be noticed that only one limb of each 'loop' lies completely within the region of the distal pole of nucleus and cell. The other end may, in some cases (the longer chromosomes), lie not far from this pole, but more usually it is in the region of the opposite (proximal) pole, or on the right or left side of the nucleus. Many investigators (Montgomery '05, Davis '08, and others) have made this mistake in attempt-

ing to find loops in this stage, where, since they were dealing with
only straight-rod chromosomes, there could be none. And in
doing this they have attempted to see in these loops, so formed,
the evidence of end-to-end synapsis, misled probably by Mont-
gomery's ('03) work on Amphibia. He, it is true, was there
dealing with loops, since the Amphibia have ten pairs of **V**'s,
and he correctly saw the constriction shown at the apices of these
loops; but these constrictions did not mean that telosynapsis
had taken place between one of the ends of each of a pair of **V**'s;
on the contrary, they probably corresponded to the constric-
tion at the apices of the spermatogonial pair of **V**'s, which were
here in parasynapsis.

In those species, however, where some **V**'s occur, as in Chorthip-
pus, one might expect a few of the loops characteristic of the 'bou-
quet' to appear. This may be seen in Davis's ('08) figure 48
of Stenobothrus. Finally, in those species where all or nearly
all the chromosomes are of this **V**-shaped type, we find the
nucleus in the 'bouquet-stage' with practically nothing but these
loops present, as the Schreiners have shown for Tomopteris
('06a, figs. 20f, 22, 23, 25, 26), and Montgomery ('03, figs. 2,
3, 5), Janssens ('05, fig. 42), and the Schreiners ('06b, fig. 12)
for the Amphibia.

I have not studied the 'bouquet' and the earlier synapsis
stages in Syrbula or Chorthippus, but only the later synapsis.
My first stage (figs. 149, 151, 163–168) corresponds, I believe, to
stage '*h*' of Wilson ('12) and stages '*f*' of Davis ('08), since it
not only resembles these, but, like them, is immediately preceded
by a stage in which no regular spireme can be distinguished,
the chromatin being in a fine netlike condition, possibly the 'dif-
fused' stage ('*g*') of Wilson ('12, figs. 66, 67).

As explained in the description (pp. 210–215), each autosome,
when it emerges from stage '*g*,' consists of two longitudinal
strands in both Syrbula (fig. 149) and Chorthippus (figs. 163–167).
These two-strand spiremes resemble very much those of Pam-
phagus shown by Granata ('10, figs. 25–27); Dissosteira, Steiroxys,
Stenobothrus by Davis ('08, figs. 57, 58, 70, 83); Ceuthophilus,
by Stevens ('12, figs. 27, 28); Oncopeltus, by Wilson ('12, figs.

107, 108); Tomopteris, by the Schreiners ('06, figs. 22, 23, 25, 26, 29–35); Salamandra, by the Schreiners ('06, figs. 10–13; '08, figs. 13–22); Batrachoseps, by Janssens ('05, figs. 57–60); Amphibia, by Montgomery ('03, figs. 2–4); and Lilium, by Grégoire ('99, figs. 5–8; '07, figs. 12–19). With the exception of Davis, Montgomery, and Granata, these authors have considered this stage to be the result of the side-to-side pairing of homologous chromosomes now beginning the disjoining process, and I agree with them.

In the later stages four strands were seen in each spireme (figs. 164–167). Such spiremes are similar to the tetrads which Carnoy described in 1887 for the nematodes Ophiostomum, Ascaris clavata, and Ascaris lumbricoides as being formed by a double longitudinal division of the single primary maturation chromosome rod, and to those seen in Ascaris megalocephala in the same year by Boveri ('87), who gave the same explanation of their origin. Granata likewise found, in Pamphagus, the early prophase chromosomes of the first spermatocyte to consist of four longitudinal parts, the result of splitting in two longitudinal planes at right angles to each other. I do not agree with him in regard to the origin of tetrads, nor in regard to the time and manner of reduction, but I am able to confirm his figures.

In Syrbula there were eleven of these spiremes, in Chorthippus, eight, corresponding respectively to the number of pairs of autosomes in each species. Polarization of the material in the spiremes was shown by the presence of the polar granules (knobs). These were terminal in Syrbula, a pair for each of the eleven chromosomes. In Chorthippus but five showed terminal knobs. The three remaining (long) spiremes each showed two pairs of the granules or thickenings, located at the point along the chromosome corresponding to the position of the apices of the V's which had conjugated to produce them. The number of knobs in Chorthippus will therefore be seen to be eleven, as in Syrbula, consisting of the five terminal pairs on the short spiremes and the two non-terminal pairs on each of the three long spiremes. In the two-strand condition (fig.

163) only two pairs of these knobs appeared; in the four-strand, four pairs. Between the two thickenings on each strand (figs. 163, 174, 176, 178) is a constriction and segmentation which is identical with the non-staining region at the apex of the V-chromosomes of the spermatogonia (fig. 162) and is similar to that which occurs in the V's of Lepidosiren and Trillium and the bipartite rods of Copepoda. This constriction divides each of the three four-strand spiremes into two unequal parts, corresponding in length with the limbs of the three respective pairs of spermatogonial V's. The facts, (1) that the position of these knobs and the constriction of the filament between them corresponds with that of the constriction and apices of the three pairs of V's, respectively, and (2) that there is among the three long spiremes but one such *un*equally segmented spireme for every pair of V's, and further (3) that such single segmented spireme cannot have resulted from a pair of V's in telosynapsis (since the segments of each of the three long spiremes are unequal) and (4) that the presence of all other spiremes in these nuclei has been accounted for, lead to the conclusion that in these three long spiremes of Chorthippus we probably have the result of a parasynapsis of each of the three pairs of V-chromosomes, which took place at some previous time. The ends of the spiremes represent the distal ends of the limbs of the V's and their knobs the proximal or apical region of these V's. If this be granted, then the autosome spiremes in Syrbula corresponding to the limbs of these V's must also be in parasynapsis, for in each case their behavior is similar to that of the V's. Since the remaining five autosomes in Syrbula and Chorthippus are similar to these three—each of them splitting into four longitudinal parts—I believe that all autosome spiremes, eleven in Syrbula and eight in Chorthippus, have united at some previous time in parasynapsis.

The remaining figures of the first maturation chromosomes of Syrbula and Chorthippus I shall, for convenience, group into four stages, '*i*,' '*j*,' '*k*,' and '*l*,' but will discuss them together. Stage '*i*,' early to middle prophase, includes figures 150, 152, 153 of Syrbula and figures 174 to 177 of Chorthippus; stage '*j*,'

late prophase, includes figures 155, 156 of Syrbula and figure 178 of Chorthippus; stage 'k,' metaphase, figures 157, 158 of Syrbula and figures 170, 180, 182a, 182b, 183a of Chorthippus; and stage 'l,' late metaphase or early anaphase, figures 181, 182c, 183b of Chorthippus, but none in Syrbula.

It will be seen that, according to Janssens, the Schreiners, and others, the side-by-side pairing of the leptotene chromosomes begins at the distal ends. In the case of a pair of **V**'s, it begins at the free (distal) ends of the limbs of the pair. From the appearance of the spiremes of the autosomes of Syrbula (fig. 149) and of Chorthippus (figs. 164–167, stage 'h' of Wilson, '12)— where in at least two cases (figs. 149, 164) all of the.autosomes are clearly visible—one is forced to admit that side-by-side pairing has taken place and probably has proceeded from the distal to the proximal part of the chromosome pair. If we grant that side-by-side pairing occurs and that it begins at the distal ends, then, if the process advances regularly, the last region of the two threads to become approximated would be the proximal ends of the rod in the case of rod chromosomes and the apices of the **V**'s in the case of **V**-chromosomes. In unequal tetrads formed by unequal homologous chromosome pairs, in Tettigidea the no. 4's (figs. 115, 120), in Acridium the no. 1's (figs. 141–147), in three genera of Oedipodinae, viz., Arphia, Brachystola, and Dissosteira (Carrothers, '14), in Schistocerca (Hartmann, '14), and in the **V**-rod bi-tetrad of Jamaicana (figs. 197–199), one feels very certain that reduction division is taking place. If this be admitted, then in all of these cases the last region of the pairing chromosome to be in contact with its mate is the distal end. Granting this, and knowing also that the first region to pair is the distal, it may be said that *the proximal regions are the last to pair and also the first to begin the process of disjoining.*

In a very large chromosome, such as 7–11 in figures 163 or 165, it is of course impossible to say whether the gaping in the middle represents the end stages of the approximation of the univalents in parasynapsis or the beginning stages of their separation in the disjoining process. I think it is the latter, because in the follicles furnishing material for figures 163 to 166

there were several cysts of cells which were in earlier stages than those drawn, probably in Wilson's '*g*' or 'diffused' stage, which is known to *follow* the approximation stage of *parasynapsis,* and also because there were no breaks in the stages succeeding figure 165 up to the metaphase. In such a nucleus as is represented at figure 168, I feel still more certain that the gaping apart is the beginning of the disjoining process, because the chromosomes are much farther along in the process of condensation. I therefore believe that in the gaping apart of the **V**-bivalents we may have *the beginning of the process of disjoining.*

In the rod bivalents of Syrbula (fig. 149) the proximal parts (x, x), which are terminal in this species, are probably in the beginning stages of the process of disjoining. This may be claimed for at least those six bivalents (5, 9; 7, 11; and 8, 10) which we have many reasons to believe are the equivalents of the three large chromosomes of Chorthippus (fig. 168). The behavior of the remaining five bivalents of figure 149 is similar to that of the larger bivalents, and therefore they are probably undergoing the same process.

First maturation chromosomes, which are probably in the end stages of approximation, some of them possibly in the beginning of disjoining (Schreiners, '06a, fig. 30), behaving like the 5–9 7–11, and 8–10 compounds of Chorthippus (figs. 163 and 165), have been described in the work of the Schreiners ('06a, figs, 22, 30; '08, figs. 16–18) for Tomopteris. Later stages shown in Salamandra (the Schreiners, '06b, figs. 12–14) are undoubtedly in the disjoining process. The gaping loops of their figures 16 to 18 ('08) are almost duplicates of 7–11's and 8–10's in my figures 163 and 165. Their figures 16 and 17 show, in addition to the loop, the halves of which gap apart in the middle, a short rod with halves gaping apart at the end. This I assume to be a rod pair; the side-to-side approximation, having started at the distal ends, is progressing toward the proximal ends, which in this case are free (not attached to another rod) and thus form a **V**. The large-loop chromosome in the same cell is possibly in the same condition.

I may assume, then, that in my figures of the '*h*' stage parasynapsis has taken place, since they are so much like those which

authors have so interpreted, and since we may be reasonably certain that in the case of the compound chromosomes of Chorthippus such has occurred. The fact that gaping-apart occurs between the two strands at the middle of the compound chromosomes, and that a similar phenomenon occurs in Tomopteris and Batrachoseps, where the Schreiners have shown that parasynapsis takes place first at the free ends of the limbs of the V's, forces one to believe that in Chorthippus likewise we may expect to have parasynapsis beginning at the free ends of each pair of V's. It seems perfectly possible, then, that in this process of simultaneous approximation of the distal ends of each pair of limbs of a V pair there may be accidental interlocking between the members of two pairs of V's, such as we find in figure 163, between the 8–10's and 7–11's, or, as appears to have been the case in the pairs shown in a later stage at figure 177, or, still better, the interlocking which the Schreiners ('06b, figs. 24, 25) have described in Salamandra. It seems to me that such figures can be explained only on the assumption that the phenomena have taken place in the manner I have set forth.

If we admit that the interlockings just described have resulted during approximation side-to-side of pairing V's, then the presence of such conditions in the stage when separation (fig. 163) is evidently beginning, means that the split appearing at the middle of chromosomes 7–11, 8–10, 5–9, must be the split of reduction, and that this stage must mark the beginning of the separation of homologous chromosomes. For, following this split through figures showing these compound chromosomes, it is evident that it gives rise to the space of the middle or perpendicular-ring portion of each compound. This middle ring may, as before said, be thought of as being formed by the four proximal knob-like parts of the two pairs, 7's and 11's, 8's and 10's, etc., which have become linked to form the compound (compare figs. 155–158 with 174–180). The points of constriction, from which the attraction fibers spring at the opposite sides of this ring, are evidence of this. The terminal portions of the compound—forming horizontal rings, if the rod chromosomes involved be long (11's, 10's, or 9's), or horseshoes, crosses,

and tetrad-rods, if the rods involved be short (8's, 7's, and 5's)—
may be considered identical with the body or main part of a
Syrbula no. 11-, 10-, 9-, 8-, 7-, or 5-tetrad of this stage. As
the metaphase passes into the anaphase and the process of dis-
junction approaches its end, the perpendicular-ring portion in-
creases at the expense of the horizontal portions (figs. 182, 183)
until, in the anaphase, the shorter members of a compound
(5's and 7's, fig. 181) separate, leaving the longer members still
attached, their horizontal rings changing to crosses (figs. 181,
182c) and finally to rods just before the completion of the dis-
junction. The circular space enclosed by the perpendicular ring
in these compound tetrads becomes, in the anaphases of the first
maturation division, the space between the V-shaped daughter
chromosomes resulting from division of these bi-tetrads. Since
it is the same space as that seen in parasynapsis between the
two gaping-apart strands of these compound chromosomes, *we
conclude that the first division must be reductional.*

Turning now to the literature involving V-chromosomes of
these stages (synapsis and reduction), I find figures of prophases
that are comparable with what I have here described for Chorthip-
pus. In the lily Grégoire's ('99) figures 6, 8, and 12 resemble
my '*j*' stage (fig. 174). His figure 19a is identical with my figures
183a and 183b, and figures 19b and 19c are similar to my 7–11's
and 5–9's in figure 181. The result of the division, if continued,
would have been an equal-armed V going to each pole (his
figs. 19b and 19c), each in turn split longitudinally in preparation
for the second maturation division (see my figs. 184–186). In
Tomopteris (Schreiners, '06a), which has many V's, I find one
of the best series of such chromosomes. Their figures 34–36
correspond to my stage '*i*,' figure 38 to '*j*,' and figures 39, 49 to
late '*k*' or early '*l*.' Upon examining their figures it will be
noticed that the perpendicular ring of the middle region increases
at the expense of the more distal portions until there is a break-
ing through of the short arm and finally of the long arm of the
V (figs. 53, 56, 57, etc.). Frequently the long arm of the V
forms a cross at the junction with its mate, similar to that of
my figure 183. In the anaphases (figs. 58–60) the V's in their

split condition pass to the poles as usual. I believe the Schrein-
ers, like most of the workers on Amphibia, have frequently
shown incorrectly in Tomopteris these compound chromosomes,
owing to a misunderstanding of the conditions. The common
error is to represent the loops at the junction between the per-
pendicular (middle) and horizontal (terminal) rings as though
they simply crossed ('06a, figs. 34–38), whereas they split and
then contribute to each side of the horizontal ring in the manner
I have shown for chromosome 7–11 in figures 173a, 174, and 176.
In the early stage 'h' it is not so easy to see the nature of these
junctions, but in the 'i,' 'j,' and 'k,' stages the figures of most
authors show very clearly that these crossings have been mis-
interpreted; e.g., Janssens ('05, figs. 58–60) in Batrachoseps,
('01, figs. 8, 9) in *Triton*; Flemming ('87, figs. 3, 4, 7a, 8) and
Meves ('96, fig. 50) in Salamandra; Grégoire ('99, figs. 7, 8) in
the lily; Atkinson ('99, figs. 1, 2, 3, 4) in Trillium. In the paper
by Agar ('11) on Lepidosiren figure 15 shows this stage, though
not very clearly.

In Copepoda the chromosomes are so short that one could
not expect to find two- and three-ring structures in the prophases
and metaphases of the first maturation, such as are found in
species with long **V**'s. But short **V**'s and the transverse seg-
mentation are found, as in Chorthippus. This point of seg-
mentation corresponds, I believe, to the apices of the **V**'s of
Chorthippus, even though the chromosome may be a rod. The
resemblance to my stage 'i' may be seen in the prophase figures
of Lerat ('05, figs. 18, 19, 39). In his figures 27–30 and 40,
(early anaphases of the first maturation) the **V** nature of many
of these chromosomes is evident in the longitudinal split of their
arms. In the first maturation prophases of Matscheck ('10,
figs. 37, 62–64, 75, 78) the paired bivalents may be considered
to correspond with those of my figures (163–168) of stage 'h.'
In slightly later stages ('i,' 'j') the transverse segmentations on
both rods of each bivalent are similar, I believe, to the constric-
tions on each of the two strands of my compound bivalents
(figs. 168, 176). These paired chromosomes, each transversely
segmented and longitudinally split, give rise to four similar

longitudinal rods lying side by side in his 'biseriale Anordnung' (see Matscheck's text figs. 10, 11,. etc.). Such transversely segmented, four-strand, rod chromosomes of copepods correspond to the transversely segmented **V** compounds of Chorthippus (my figs. 164, 165, 168, 176, etc.). In anaphase the spindle fibers, evidently attached to the constricted part in the middle, make the daughter chromosomes appear **V**-shaped (his text figs. 7, 8), as in Chorthippus. In synapsis they remain in contact longer than the intermediate parts of the chromosomes. I believe, in short, that the "chromosomes in biserial arrangement" (Haecker and Matscheck) are indeed compound chromosomes.

Those investigators of insects who have worked upon species of Stenobothrus and of the Locustidae have found a few of these chromosomes. Stevens ('12) shows for Ceuthophilus two chromosomes (fig. 29) similar to my figure 176, but, as usual, has misrepresented the relation of the middle to the terminal rings at the points of crossing (figs. 30a, 30b). In the metaphase and anaphase (figs. 31–33) there are evidently **V**'s with unequal arms separating from each other. One of them shows the **E**-appearance. In Stenobothrus (Chorthippus) de Sinéty ('01) has shown, but again wrongly interpreted (figs. 122, 123), these compound chromosomes. Compare *b* in his figure 124 with my no. 8–10 chromosome in figure 178; also *a* in figure 125 with my figure 182c. The latter he has illustrated incorrectly, I believe. Davis ('08, fig. 87) gives two chromosomes similar to my no. 7–11 in figure 174. He is, like others, wrong in showing the loops of the middle ring crossing each other rather than passing into each loop of the terminal rings. What I have found in this species (figs. 181–183) is almost identical with his figures 88, 91. The criticisms I have made of Davis's figures applies to those of Gérard ('09, figs. 37–44, S. biguttullus) and of Meek ('11, figs. 10, 11, 14–16, 18; '12, figs. 276–289) in S. viridulus.

In the works of Baumgartner ('04) and Payne ('12) on Gryllidae, which exhibit a large number of **V**-chromosomes, the peculiar perpendicular rings of their metaphases probably result from the separation of the members of **V** pairs. Nowlin ('06) deals in Coptocycla with many pairs of **V**-shaped chromosomes.

The loops of the 'bouquet-stage' result from the pairing of V's. The points on these which, following Montgomery ('03), she thought were points of junction of homologous chromosomes in telosynapsis, I believe were constrictions at the apices of V's in parasynapsis. In the metaphase her perpendicular rings and E-shaped figures are the result of the separation of V's in reduction, as in Chorthippus.

In Amphibia Flemming ('87) figures (fig. 23), I believe, a three-ring chromosome of stage 'j,' like my no. 7–11 of figure 174. In the first maturation division the majority of his chromosomes (figs. 21–25) are in the perpendicular-ring condition—a late 'k' or early 'l' stage (my fig. 183b). Many show the cross-form on the side of the ring which is derived from the longest arms of the V's concerned in making the ring, as in Chorthippus. In anaphases (stage 'l') of these chromosomes (figs. 26, 28) the V's—unequal-armed, equal-armed, and hook-formed—are each split longitudinally in preparation for the second spermatocyte division. This phenomenon suggested to Flemming the name 'heterotypical' for this division. For Salamandra the Schreiners ('06b) have shown in figures 13 and 14 the 'i' stage, though it is incorrectly represented with loops crossing; at figures 15 to 20, the 'j' stage; and at figures 22 to 25, the perpendicular rings in late 'k' stage, similar to my figures 183a and 183b. In figure 27, at the left, is a perpendicular-ring chromosome with horizontal ring almost identical to my figure 182c.

Montgomery ('03) saw in Amphibia the constriction at the apex of V's in parasynapsis (stage 'h') similar to my figures 163 to 165, 168, but interpreted this constriction as the point of junction in telosynapsis of two spermatogonial chromosomes. I believe that he wrongly identified this point of constriction with the point of separation between the *distal* ends of the limbs of the V's, which, in the final stages of disjunction, form, as in Chorthippus, the long perpendicular rings of the first maturation, late metaphase. In other words, he thought the point of segmentation at the apex of the V's in parasynapsis was the same as the ends of the limbs of the V's in the metaphase (see his fig. 8). His final results were correct, but his interpretation of the stages

from the 'bouquet' up to the metaphase was wrong. His mistake has caused no little confusion in the work of younger investigators.

With the exception of the workers on copepod material, and of Agar (Lepidosiren), no one thus far cited has shown in the first maturation chromosomes resulting from V-type pairs segmentation at the apices of the separating halves of the perpendicular rings, such as may be seen in figures 174 to 183 of Chorthippus. In Lepidosiren Agar ('11, figs. 27, 28, 30; '12, fig. 13b and c) has illustrated this segmentation in his perpendicular rings of the '*j*,' '*k*,' and '*l*,' stages. The V's of Lepidosiren have such short arms, however, that there results from them (when all parts of the pair of V's except the extreme distal ends have gone through the disjoining process) a four-part ring-tetrad, similar to what vom Rath described for Gryllotalpa. We can now understand the peculiar figures which vom Rath gave, for Payne ('12) has shown that in Gryllotalpa there are many V's.

2. *Is there pre-reduction or post-reduction in autosome tetrads?*

In a former paper (Robertson, '08, on Syrbula admirabilis) I held that an end-to-end synapsis of homologous chromosomes took place, that the first spermatocyte division was a longitudinal division of each of the conjugating members of a pair, and that the second division was the true reduction division. In that work, so far as the actual observations are concerned, I was correct and the observations will stand. My interpretation of the results, however, was incorrect.

In the first place, I did not make a study of the early stages of the spermatocyte, where parasynapsis has been found to take place. My conclusions in favor of telosynapsis were based, I now find, upon what is really the end stage of parasynapsis. I have been forced to the present position by the study of the V-chromosomes in Chorthippus in comparison with those of Syrbula acuticornis. The first spermatocyte metaphase (figs. 179, 180) of Chorthippus shows in the compound chromosomes the 7 and 11, the 5 and 9, and the 8 and 10 parts behaving so much like the same (but independent) tetrads in Syrbula (figs.

157, 158), that one cannot avoid believing them identical, though they are compound in one genus and simple in the other. The connections at the apices of the **V**'s, where the spindle fibers become attached, apparently make no difference in the behavior of the rod limbs of the **V**'s in the processes of conjugation and disjunction. The limbs of the 7–11 pair of **V**'s may form rods, crosses, horseshoes, or rings of various sorts just as readily as if they were separate chromosomes. The compound nature of the large chromosomes was evident. Having this in mind, and knowing that in parasynapsis the first parts of chromosomes to pair are the distal ends (in the cases of **V**'s the ends of the limbs), my opinions in regard to the time of reduction were seriously changed when I found the interlocking that I have described and shown in figures 163 and 177. It was then evident to me that the gaping split between the halves in the middle of paired loops was, most likely, the space between chromosomes which had paired. Following this split through the series of prophase chromosomes up to the first maturation division, I found it to be the primary split and therefore the reductional division. In both species of Syrbula the prophase tetrads, consisting of rods, crosses, horseshoes, and rings, appear and behave just like the 5, 7, 8, 9, 10, and 11 parts of the compound chromosomes of Chorthippus. There is good reason to believe that in Syrbula admirabilis, as in Chorthippus, the first maturation division is reductional. I therefore must admit that in my paper on Syrbula I was wrong in regard to the absence of parasynapsis and to the time of reduction, and that, so far as concerns the time of reduction, I shall have to differ with McClung and those of his students who have taken the same stand upon this question.

A second case in which it seems to me that the first maturation division is clearly reductional is that of the single **V**-chromosome of Jamaicana subguttata (my figs. 198, 199), which separates from its two unequal rod-mates in this division. Spermatogonia of this animal show thirty-two rod-shaped autosomes in addition to one slightly unequal armed **V** autosome and the large sex chromosome. The first maturation division (fig.

197) has, in addition to the long sex chromosome, fifteen rod tetrads, plus the **V** (14, 14, 16, 16) with limbs double the length of the spermatogonial **V**. This **V** showed considerable inequality in the lengths of its limbs; moreover, there were constrictions (figs. 198, 199) at the middle of each limb, indicating the points at which separation in the last stage of disjunction is about to take place. It is probably a bi-tetrad, for, counting it as such, we get the seventeen tetrads characteristic of the first spermato-cyte. Anaphases (Woolsey, '15, figs. 44–47) proved that separa-tion did take place at the points of constriction on the double-length arms of this **V** bi-tetrad. To one pole went a **V** with unequal arms, similar to the spermatogonial **V**; to the other pole went two rods of unequal length, similar to the limbs of the **V** from which they had separated. I can think of no clearer demonstration than this, that, in so far as these chromosomes are concerned, the first division is reductional. The remaining fifteen tetrads behave in all respects similar to the limbs of the bi-tetrad **V** (figs. 195, 198, 199). In view of the fact that this **V** separates from its rod-mates (fig. 199) and that in the two-**V**-chromosome of the first spermatocytes (fig. 201) of another spe-cies (J. unicolor) the **V**'s open out into an unequal-sided ring of the same size as the bi-tetrad **V** of the first species (J. sub-guttata), there is strong reason for believing (1) that the three **V** pairs of Chorthippus likewise divide reductionally in the first maturation division; also (2) that all *perpendicular* rings of first maturation divisions resulting from **V** pairs of chromosomes are of a similar nature, dividing reductionally. Furthermore, since the limbs of the long **V** bi-tetrad (figs. 197–199; Woolsey, '15, figs. 39–43) are similar in every respect to the other tetrads present, I suspect that this division is reductional for all of the pairs of chromosomes in this genus.

A third case that forces me to take sides in favor of pre-re-duction is that of the separation in the first maturation division of the members of unequal homologous chromosome pairs, such as I found (Robertson, '15) in one specimen of Tettigidea parvipennis (figs. 115, 120, 122, no. 4's) and in one specimen of Acridium granulatus (figs. 141–147, no. 1's). From a study

of the chromosomes of a large number of individuals belonging to species of both these genera, I became acquainted with the form, the relative length, and the behavior of what appeared to be the normal chromosomes. I was able with little difficulty to distinguish the members of a pair from each other, by means of their length. When an individual was found showing one chromosome in a pair of slightly abnormal length, I was able to determine to which pair of chromosomes it belonged, whether to the 4's, or 1's, etc. This enabled me to trace its course in the maturation division. As a result I found that it separated from its mate in the first spermatocyte division, and divided equationally in the second. On passing to the pole of the first division these abnormal members showed that they were split longitudinally in preparation for the next division (no. 1 in fig. 147), which was equational. The length of the abnormal chromosome in the late metaphase, as the result of the constriction of the tetrad, or in the anaphase after separation from its mate, agreed in each case, when compared with the rest of its fellows, with the length of the chromosome in the diploid divisions. This may be seen by comparing the deficient 4's (4–) in figures 115 and 104 or 105, and likewise by comparing the abnormally long no. 1's in figures 141 to 147 with those in figures 136 to 140.

The above are not the only instances of unequal homologous chromosomes separating from each other at this division. Hartmann (March, 1913) was the first to recognize—in the primary spermatocytes of Schistocerca—what he termed 'unequal divisions' of tetrads. Carrothers later (December, 1913) likewise described a large number of cases of the unequal division of one tetrad among the three smaller pairs of chromosomes in the twenty-three-chromosome grasshoppers Arphia simplex, Dissosteira carolina, and Brachystola magna. There are probably additional divisions of this kind among the larger tetrads of these grasshoppers, but it will be more difficult to recognize them on account of their greater lengths, a slight variation showing less easily in a long chromosome pair than in a short one.

These unequal tetrads behave very much like the tetrads of the two other small pairs of chromosomes of these twenty-three-chromosome grasshoppers. If we may assume specific individuality for the members of each of the twenty-two pairs of autosomes in the subfamilies of this group of grasshoppers, then we may draw the conclusion that the tetrads of at least seven of the eleven pairs of autosomes divide reductionally in the first maturation division. This number is reached by counting Carrothers' ('14) one small pair and the six pairs associated in the **V**'s of Chorthippus, leaving out of the enumeration the **V**'s of Hesperotettix and Mermiria.

A fourth case which supports my contention is that of the multiple **V**-chromosomes of Hesperottetix and Mermiria, just mentioned, which McClung ('05, '14) found to divide reductionally in the first maturation division. In Hesperotettix one **V** occurs in the spermatogonial divisions. One limb of this **V** is the sex chromosome, the other limb an autosome. In the first spermatocyte the rod-mate of the autosomal arm becomes paired with that arm and may be seen separating from the **V** in this division. Something similar occurs in Mermiria, except that there are two autosomes, instead of one in the form of a **V**, associated with the sex chromosome. The autosomal part of this compound probably separates from its autosomal **V** mate in the first maturation division.

From the many instances which I have here given, it seems to me the inference may possibly be drawn that all autosomal tetrads will be found to divide reductionally in the first maturation division.

3. The question of synapsis

1. Is synapsis a fact? Do chromatin elements actually conjugate or otherwise become associated two by two? I am assured of the occurrence of synapsis from the behavior of the **V**-chromosomes of Chorthippus, of Jamaicana and of Hesperotettix, and from the behavior of the unequal homologous chromosomes of Tettigidea parvipennis, Acridium granulatus and other species.

In Chorthippus my conclusion is based in particular upon a comparison of its total chromosome complex, V's and rods, with (1) the total rod series of its nearly related genus, (2) the series of its subfamily, and (3) the series of two related subfamilies. This comparison enables me to locate certain points upon the members of three pairs of chromosomes in Chorthippus, and by that means to identify them in the bivalent chromosomes of the first spermatocyte, at least from the stage which Wilson has termed '*h*' up to and through the two maturation divisions to the spermatids.

I find that all the genera of the subfamily to which Chorthippus belongs, with the exception of a single species, the identity of which is not certain (McClung, '14), have eleven pairs of autosomes. These autosomes, so far as length is concerned may be arranged in three groups: (1) a series of three very short pairs, (2) five intermediate pairs forming a graded series among themselves, and (3) three very long pairs (Robertson, '08, pls. 21, 22; present paper, figs. 148, 157 to 162, 178 to 180, 184 to 192). I find the same number of chromosomes, and to a certain extent, the same size relations in the Oedipodinae and Acridinae. If, therefore, for these three subfamilies of the Acrididae there is such a constant number of chromosomes and also, to a certain extent, of sizes, then it is reasonable to regard each of the eleven pairs of autosomes as a pair of similar individual organs in the cells of every species belonging to the genera of these subfamilies. That established, it is possible to trace these recognizable pairs of autosomes through different genera, whether they be separate, as in Syrbula and most genera, or linked together, as in Chorthippus. Syrbula and Chorthippus are two closely related genera—one has no linkage in its chromosomes, the other has three pairs of V's. As I have explained at pages 235–237, the only conclusion to be drawn, in the case of the V-chromosomes at least, was that during the synapsis period they had paired two by two.

A second line of evidence that synapsis takes place is afforded by the behavior of the V's in Jamaicana, where one individual showed in its first spermatocyte cells a single unequal-armed

V autosome paired with, and separating from, rod-autosome mates (pp. 221–222, 246–247). In germ-cell divisions preceding synapsis the V autosome existed separate from the rod autosomes, but in divisions succeeding synapsis it separated again from them, passing into one-half the spermatids while the rod autosomes passed into the other half. I can think of no clearer evidence than this that synapsis takes place—that chromatin elements actually become associated two by two.

In the unequal homologous chromosome pairs of Tettigidea parvipennis and Acridium granulatus, we have a third line of evidence for synapsis. In one case an abnormal individual was found having a deficient no. 4 chromosome paired with a no. 4 of normal size (figs. 115, 120); in an other case, an abnormally long no. 1 paired with a normal sized no. 1 (figs. 136–147). I find that the members of these unlike pairs in the two individuals exist apart in diploid series (figs. 136–140), together in synapsis in the first spermatocyte of the germ cell generations (figs. 141–143), and that they again separate from each other at the first spermatocyte division (figs. 144–147).

2. Admitting the fact of synapsis, are the conjugating elements chromosomes, and are they individually identical with those of the last diploid or pre-meiotic division? To prove this, one must be able to trace the individual chromosomes from the telophase of the last spermatogonial division to the prophase of the first spermatocyte division. I have not done this in the cases described in this paper. My evidence must, therefore, be indirect.

Giglio-Tos ('08) and Granata ('10) look upon the chromosomes as being not permanent morphological structures, but only temporary formations resulting from chemical processes of some sort. They therefore believe that the individual chromosomes become disintegrated and disappear after the last spermatogonial division, that the chromosomes of the first spermatocyte are formed anew in half the former number, each split twice longitudinally, thus becoming tetrads for the maturation divisions. Meves ('96, '07a), Fick ('07, '08) and Duesberg ('08) likewise believe that the individuality of the spermatogonial chromosomes is lost during this period. According to

their views, there is no such thing as conjugation two-by-two. A continuous spireme is formed anew in the nucleus, and breaks up into half as many segments as there are chromosomes. These segments divide twice longitudinally and are distributed during the two maturation divisions to the spermatids. Both maturation divisions are considered to be equational.

The theory of the Italians cannot be discussed in this connection, since they deny that chromosomes are morphological structures. According to the views of Meves, Fick and Duesberg, it would be hard to explain how, in a nucleus like that of the one-V-type individual of Jamaicana subguttata, a continuous spireme could break up into just half the number of segments, one of which is destined to give rise to the peculiar V-bi-tetrad (figs. 197–199). This segment in the first place would have to split longitudinally, the daughter threads separating along their whole extent except at the ends (figs. 201, no. 14–16), which would remain in contact up to the beginning of the anaphase. In the meantime, before separation is completed at the ends, one of the daughter threads would have to divide transversely at a point not in the middle of the strand to give segments in length equal to a no. 14 and a no. 16 chromosome (figs. 198, 199). This would have to be repeated accurately in all cells of the first spermatocyte. It is highly improbable that such could occur. The theory fails to explain the phenomenon of reduction in numbers or the formation of this peculiar type of first maturation chromosome.

In the case of the unequal homologous chromosomes of Acridium and Tettigidea, the fact that the members of the pair separate from each other in the first maturation division and are distributed each to only one-half of the germ cells is proof that the zygotes of the next generation will be formed with varying combinations of these chromosomes in their nuclei. It is difficult to imagine a continuous spireme being formed in the growth period of the first spermatocyte which should become divided so accurately that it would give rise to segments of the exact length of these abnormal chromosomes which are characteristic for this particular combination and seem to be present in *all* cells of the

animal resulting from such a combination. At the formation of zygotes, combinations must have been made having, for instance, either an abnormally long no. 1, and a short no. 1 (figs. 136–140, 141–147) or two short no. 1's (the most usual); and, in the case of the no. 4's, one normal and one deficient no. 4, or two normal no. 4's. While such a segmentation must be uniform for every cell division in this animal as a result of Mendelian segregation and recombination, the same chromosomes in the next generation will have to give rise to an entirely different segmented spireme at each division. This cannot be imagined. The same may be said in respect to the V's and their rod-mates in Jamaicana, where we have undoubted evidence of Mendelian segregation and recombination, since in the same species there were individuals having two pairs of rods, and again two rods and one V, or in another species two V's. It seems to me that it will have to be admitted by Meves, Duesberg, and others that these abnormal chromosomes persist from one cell division to the next. In order to do this they must remain independent of each other and of the mates with which they pair, for pairing in this case is proved to take place. They must be identical from cell to cell of the same animal, for in the one-V type of Jamaicana subguttata every cell, except one-half the second spermatocytes, possessed this V-chromosome. The same may be said of any of the other abnormal chromosomes. These chromosomes persist as entities from one cell division to another and especially from the pre-meiotic to the first spermatocyte division; otherwise how can we explain the repeated appearance of such irregularities in so exactly similar a manner in all dividing cells of the animal in which they happen to be?

3. *Do they conjugate side by side (parasynapsis, parasyndesis) or end to end (telosynapsis, metasyndesis) or in both ways?* The only evidence I have to offer that is of any force in answer to this question is that afforded by the V-chromosome compounds in Chorthippus. As has already been shown (pp. 232–234), they give strong evidence in support of the parasynaptic method of conjugation.

4. Does synapsis lead to partial or complete fusion of the conjugating elements to form 'zygosomes' or 'mixochromosomes,' or are they subsequently disjoined by a reduction division? Bonnevie ('06, '08) and Vejdovsky ('07) believe that parasynapsis occurs and that in this process a complete fusion takes place, which is permanent. As a result, a new chromosome is formed, two longitudinal divisions occur, and a four-strand first-maturation chromosome results. Both first and second maturation divisions are simply equational, not reductional. Haecker in his metasyndesis theory holds a somewhat similar view, with this difference, however, that as a result of incomplete (only half as numerous) transverse segmentations of a continuous spireme, previously formed by the union end to end of the chromosome threads, there appears just half the number of segments (reduction), in which homologous spermatogonial chromosomes find themselves united end to end. This union is conceived to be permanent and evidence of it may be seen, according to Haecker, in the transverse segmentation so often visible in chromosomes of the copepods during both first and second maturation divisions as well as in the division of soma cells. Both maturation divisions are, according to Haecker, equational, merely dividing his two-part rods longitudinally into four similar two-part rods to be distributed to the four spermatids. It will be seen that no reduction or segregation in the usual sense of the term occurs. The germ cells, then, all receive the same sort of chromosomes.

In view of our work upon the transversely segmented chromosomes in Chorthippus and Jamaicana, whose segmentation we have found to be due to the usually permanent association of two non-homologous chromosomes, we have many reasons to believe that Haecker has probably been dealing with similar cases of compound chromosome formation. The work of Lerat ('05), Matscheck ('10), Krimmel ('10) and others has shown the presence in copepods of chromosomes in the form of V's with, in many cases, unequal arms, as well as the usual segmented copepod rod, which likewise in some figures has unequal instead of equal segments. These observations agree with mine upon

the V's of Chorthippus and Jamaicana, in which no pairs have arms of equal length. These conditions make it improbable that the V is a pair of homologous chromosomes in telosynapsis. It is self-evident, that neither the separation from each other of unequal homologous chromosomes in Tettigidae nor the separation of the V from its rod-chromosome mates in Jamaicana subguttata during the first maturation division, can be explained according to Haecker's metasyndesis theory.

In regard to the complete fusion of homologous chromosomes during parasynapsis, as held by Bonnevie and Vejdovsky, I have again some very important evidence in the unequal homologous chromosomes, and likewise in the V's of Jamaicana and Chorthippus. I have discussed in full with figures (Robertson '15, plate 3) in my Studies III the evidence afforded by the unequal pairs. In Chorthippus the interlockings which I have found between the no. 8–10 and 7–11 compounds (fig. 163) and between the 5–9 and 7–11 compounds during the middle and late synapsis periods, together with two cases that have been illustrated by the Schreiners ('06b, figs. 24, 25), furnish strong, though rare, evidence that these pairing chromosomes during the parasynapsis period have maintained their individuality as chromatin threads. I am convinced by my work upon the Tettigidae (Study II) that parasynapsis is a fact in grasshoppers. The three long asymetrically segmented spiremes in Chorthippus, as I have already explained, furnish indirect evidence that parasynapsis must have taken place. If we accept the evidence of the Schreiners and of Janssens, side-to-side pairing probably begins at the distal ends of the limbs of a V pair. This takes place, according to them, previous to, or about the time of, the bouquet period and soon after the last spermatogonial division. It is apparently possible that the interlockings between V pairs which I have described might have occurred at this time. Following this period of side-to-side approximation, is a period of intimate side-to-side pairing, which passes into a period, the 'g' stage of Wilson, in which individual spiremes cannot be made out. Yet we find the spiremes coming out of this period in the reduced number and in the same relative sizes as are the auto-

some series of pairs. Now, apparently, after going through all of these stages, these spiremes exhibit interlockings between pairs of **V**'s, such as the Schreiners and I have described; interlockings which I believe must have been made at the beginning of parasynapsis. This to my mind is of significance, and means that these pairing chromosomes probably maintain their individual identity while passing though these stages, and that there is probably not so intimate a fusion of them as to necessarily result in loss of continuity as a thread.

Further evidence that parasynapsis probably does not result in complete fusion of the pairing chromosomes can be drawn from the **V**-rod pairs of Jamaicana subguttata, providing we assume parasynapsis to have taken place in this double pair (figs. 197–199; also Woolsey, '15, figs. 37–46). If we make this assumption, then in the process of disjunction the proximal ends of the rod-mates have evidently rotated away from the proximal region (apex) of their **V** mate until each of the rods has come into line with its respective limb of the **V**, as is shown in most of the figures (fig. 37 especially). All division figures found in which this **V** bi-tetrad could be studied gave distinct proof that the length of the no. 16 rod corresponded with the length of the no. 16 limb of the **V**, and the length of the no. 14 likewise with its limb of the **V**. In all such divisions this long bi-tetrad **V** with segmented limbs was the only form in which the body appeared. This is evidence to my mind that these rods in separating from their respective limbs of the **V** have separated along the plane in which the side-to-side pairing took place. Otherwise, if a complete fusion of these rods with the limbs of the **V** had taken place with possibly splitting in a new plane, there would have resulted a variety of forms of this compound body ranging from the long bi-tetrad **V** form we have here to an elongated unequal sided ring, such as is found to be constant for the species Jamaicana unicolor (fig. 201; Woolsey, '15, figs. 65–67). The constancy with which this **V** separates from its rod-mates in unmodified form is strong though indirect evidence that parasynapsis, if it occurred, was not as complete a fusion as Bonnevie and Vejdovsky would maintain.

4. On the chiasmatype of Janssens

In the first spermatocyte chromosomes of Syrbula and Chorthippus (figs. 150, nos. 6, 8, 11; 152a; 156b; 174, 7–11; 176; 178) I have bodies similar to those of Triton and Batrachoseps upon whose structure Janssens ('09) bases his 'theory of the chiasmatype.' Morgan ('13, '14) has made much use of this theory in explaining the failure of the linkage of characters usually coupled together in transmission to offspring. Janssens used it to furnish a basis in the germ cells to account for the occurrence of a larger number of allelmorphs than there are pairs of chromosomes by which they may be borne.

According to this theory the chromosomes which pair side by side in synapsis twist about each other in an irregular spiral manner on coming out of this stage. Before the members of a pair separate each may be seen to be split longitudinally, so that the tetrad is made up of four longitudinal strands. Janssens has noted strands crossing over from one conjugant chromosome to the other (his text, fig. XXII) and upon the basis of this observation concludes that when such paired chromosomes later complete their disjunction they represent combinations different from those present before synapsis due to the establishment secondarily of these 'cross-over' connections.

If Janssens assumes that the breaking and secondary fusion of those crossed-filaments nearest each other (text figs. XIII–XV) takes place after the chromosome has reached the stage represented by his figures 1 to 13 (pl. I) etc. (stage 'h' or 'i' of Wilson), I believe that he has been mistaken in his interpretation of the form of the chromosome (text fig. XI) upon which he has based his theory, but if he believes this secondary fusion takes place much earlier, following the amphitene and preceding the pachytene stage, then I have little to say. As will be seen from his figures (text figs. I, IX, XI, XXI, XXVII), he believes that the chromosomes conjugating side by side (each split longitudinally) should normally cross each other entire, i.e., with both filaments, at the nodes (N, text fig. I). He finds, however, that instead of both filaments of a segment

(portion of a conjugant between the nodes) crossing over together to the next segment, only one may cross (text figs. XII, XXII). This he assumes to mean that a single original filament (longitudinal half of a conjugant) of each chromosome has broken at the point of their crossing (text fig. XIII) and that broken ends of one filament have traded connections with the broken ends of the other (text figs. XIII–XV, XXII, XXVII). That it is unnecessary to assume this, is shown by the fact that we are able to trace all steps in the formation of such 'cross-overs' in tetrads of the ring and cross type back to the stage in which the tetrad consists of a rod split in two longitudinal planes, and consisting of four longitudinal filaments lying side by side (figs. 150–156, 164–168, 173–178). The crossing over of filaments results from the tendency of the four-strand rod to split part of the time in the primary plane (I), and part of the time in the plane at right angles to it, the secondary plane (II). If the chromosome be short, but one cross-over occurs and 'a cross' results (nos. 6 and 8, fig. 150; fig. 156c); if the chromosome be long, two, or, as in the compound chromosomes of Chorthippus, even three cross-overs may occur (figs. 150, 10's and 11's; 155, 6's, 9's, 10's and 11's; 156d–156f; 174, 7–11's, 5–9's, 8–10's; 175, 176, and 178, 8–10's, 7–11's). This formation of the cross-overs as a result of the opening-out process of the four strands does away with the possibility of a "compénétration graduelle de deux chromosomes au niveau d'un chiasma avec la soudure des filaments qui se touchent les premiers," as Janssens has supposed according to his text figures XIII–XV.

Many investigators who have worked upon species in which there occurred tetrads resulting from these long V chromosomes (Janssens, '05 among others) have misinterpreted their form, representing them as two spirals twisting about each other (Janssens's text fig. 1, page 391, or Davis's, '08, figure 87), rather than in the way I have shown them in figures 156f, 174 (7–11's and 8–10's) and 176. In like manner, when dealing with shorter chromosomes which give crosses (my figs. 150, 6's and 8's; and '08, figs. 29c, 29h) and simple rings having at one point on the circle (sometimes an additional pair at the opposite point)

a pair of proximal knobs or arms (my figs. 156d, 156e; '08, figs. 29b, 29f, fig. 30, no. 11, fig. 32, nos. 7 and 9), these investigators have represented them as crossing each other in the manner of Janssens's text figure XXI for crosses, or as Davis ('08) has represented at figures 179, 180 for the simple knobbed rings. In some cases I admit that a free crossing resulting from a twisting of the chromosomes, rings, etc., may have occurred, but I am certain, especially in the case of the long chromosomes of Stenobothrus, that many of these so pictured crossings do not occur, and that the chromosomes have been misrepresented in illustrations (e.g., Davis, '08, figs. 86, 87, 183–186; Meek, '11, figs. 10, 14–16, 18; '12, figs. 276–279; de Sinéty, '01; Gerard, '09).

I do not believe that the breaking and secondary fusion of crossing filaments which Janssens has postulated can take place after, or at the time of, stage '*h.*' If they take place at all, it must be in the zygotene or pachytene stage, when the pairing threads twist about each other more or less intimately and for a time appear fused. That such breaking and secondary fusion as he postulates occurs, I am not quite ready to admit, in view of the evidence to the contrary given by the unequal homologous chromosomes of Acridium and Tettigidea ('4s in figs. 115, 120, 122, and 1's in figs. 141–147) and by the V-rod tetrad of Jamaicana (figs. 197–199). In Tettigidae I am certain (Studies II) that parasynapsis takes place. Now, if there be such a fusion as Janssens says occurs in the early strepsitene stage, we should except to have irregularities in the lengths of the exconjugating chromosomes shown at the late metaphase and anaphase of the first maturation division (4's in figs. 115, 120, 122 and 1's and 1's in figs. 141–147). There is, however, no visible variation in the relative length of these separating chromosomes. This matter I have taken up also in Study III.

Something similar may be said of the V in Jamaicana, which, if we assume that parasynapsis occurs, we have every reason to believe must be paired with two rods (nos. 14 and no. 16) during such a stage as the pachytene or strepsitene. If fusions take place of the type Janssens supposes, we should expect the occurrence of irregularities in the form of the exconjugating mem-

bers of such a pair, but apparently no irregularities do occur. The **V** travels to one pole and its rod-mates to the opposite pole. They separate in the form they had on entering the pairing process. However, this is not contending that there is no possibility of a break at the apex of the **V** member of this bi-tetrad and a relinkage of one limb of the **V** with one of the free rods or with a limb of an opposite **V**. If such occurred, it would, of course, be a 'crossing over' carrying a large group of factors.

I wish to call attention to some of the peculiar mitosis figures which Janssens gives in support of his theory. The gaping apart of the distal ends of halves of exconjugants, which he believes to be abnormal (text fig. XXIII, XXIV and figs. 40, 41a, 41b, 47, 48, 50–52), is in reality not unusual for chromosomes of the Tettigidae and Acrididae. The twistings shown in anaphases, such as his figures 17, 18, 24, 30, 31, may be explained as the result of misfortune in the prophase. Frequently I find such a chromosome as figure 17 in various prophase periods from late '*i*' up to the metaphase in the same condition, the only difference being in the degree of condensation. In stages '*h*' and '*i*,' when such a chromosome is in the long-spireme condition, split into four strands and coming out of disjunction, it is to be expected that the four strands composing it will get twisted into all sorts of positions before turgidity (rigidity) sets in as a result of the condensation process. That, however, it seems to me is no argument for the breaking and re-fusing of any two of these filaments. I have similar chromosomes in no. 11 of figure 155b and 7–11 in figure 175.

But my chromosomes 5–9 in figure 178 cannot be explained as the result of a twisting in the disjunction process. Janssens has shown in his figure 14 a chromosome of the same nature. His chiasma-type theory, it seems to me, fails to explain the persistence of such twistings. It seems to me that this might be explained more readily if the splitting of the conjugating chromosomes and a twisting were to occur in each conjugant before side-to-side pairing takes place. Such might be expected in compound chromosomes of the **V** type in which pairing possibly begins simultaneously at the distal ends of both limbs of the

V's. It might also be imagined that only the ends of such split compound chromosomes should conjugate and not the mid-regions. That such *pre*synapsis splitting is possible, we are led to suppose from the reports of Dehorne ('11) and Schneider ('10) in somatic mitoses and Brunelli ('10, '11) in last spermatogonial mitoses, who found that the telophase chromosomes on their way to the poles show longitudinal splitting. Before I knew of any of these works (thus eliminating the chance of error by suggestion), I had found similar conditions in the anaphases of both somatic cells and the last spermatogonial divisions in the Tettigidae. My conclusions from the Tettigidae are, that these spermatogonial chromosomes on entering synapsis are already split. This may be seen, in each of the chromosomes of cells where the full diploid number exists. Before synapsis the split disappears. The twelve autosomal spiremes, pairing two by two, are seen to form six threads. Now, if this be the case it seems to me that we can explain such conditions as Janssens has shown in figure 14 and I in figure 178, no. 9, where one conjugant before conjugating with its mate seems to have twisted, once in mine and several times in his. In my opinion Janssens's chiasmatype theory does not explain satisfactorily this phenomenon, while that of a *presynapsis splitting* does.

This theory appears to me based upon an unnecessary and incorrect interpretation of the first spermatocyte chromosomes. It fails to explain all of the phenomena of twisting in these chromosomes, and there is some evidence to show that it probably could not occur. Until the theory can be established upon a more firm basis cytologically, we are not justified in accepting it unreservedly in our study of problems of heredity.

5. Linkage as shown in **V**-chromosomes—a basis for coupling and repulsion

Such linkage of chromosomes as Chorthippus reveals when compared with Syrbula and as the species of Jamaicana, more especially J. subguttata, show, furnishes a second possibility

of accounting for some of the phenomena of coupling and repulsion and the deviations from this, i.e., failures of linkage. Janssens's ('09) theory of the chiasmatype is the first explanation that has been offered. Phenomena of coupling and repulsion have been observed by Bateson and Punnett ('11), Correns, Baur, Emerson, East, Trow, and especially by Morgan and his students in their bred Drosophila ampelophila. In their experiments the last mentioned authors have found that certain groups of characters, usually carried together, occasionally become broken into two groups. A specific case is afforded by the results obtained when a gray, red-eyed female is crossed with a yellow, white-eyed male (Morgan '13, p. 88). The factors for gray body and red eye are usually carried together with the sex determining chromosome, their allelomorph yellow body and white eye likewise in a sex chromosome. The offspring in F_1 are all gray-bodied and red-eyed in both sexes. In the F_2 generation the expected results, assuming for the moment free interchange of the factors for gray and yellow body and for red and white eye, would be 4GR ♀ : 1GR ♂ : 1GW ♂ : 1YR ♂ : 1YW ♂. But this ratio is not realized. The results obtained are 170GR ♀ : 84GR ♂ : 1GW ♂ : 1YR ♂ : 84WY ♂, which show that there is a tendency for the factors that entered together, gray body and red eye, and yellow body and white eye, to remain together. By these numbers in F_2 it may be seen that coupling was not complete but failed in the proportion of 1 : 84, in other words the chances are 84 : 1 that the factors entering together will remain together. Now, it seems possible that such a small departure from normal coupling as 1 : 84 might be explained by an occasional break in the **V** sex chromosome at the apex. Stevens ('08, figs. 61, 65–73) gives two **V**-shaped sex chromosomes, one a normal **V** and the other carrying attached to its apex a rod-shaped element, the nature of which is not clear. That **V**'s may be formed by the fusion of non-homologous rods by their proximal ends and also that rods may be formed by the breaking of a **V** at its proximal end, the apex, is to be inferred from the presence of a **V** and its rod-mates in one and the same individual (figs. 196–199). The same may reasonably be expected

to occur in Drosophila ampelophila. Is it not possible that the extra rod-like appendage to the apex of one of the **V** sex chromosomes described by Miss Stevens may be a rod segment of some **V** that had previously broken at the apex?

Besides these sex-linked factors, Morgan has found "other factors to cross over to various degrees; in the extreme cases the chance is one to one that they cross over." Metz has shown for Drosophila ampelophila two pairs of **V**-shaped chromosomes in addition to two rod-shaped and two *M*-chromosomes. While he has not shown variation from this configuration in D. ampelophila, he has shown it in other species of the genus, where it is perfectly clear that either one pair of **V**'s may be broken up into two pairs of rods, or, similarly, both pairs of **V**'s, making four pairs of rods. While he has not shown a single **V** alternating with a pair of rods, as occurs in Jamaicana subguttata, I feel reasonably certain that it will be found. It seems likely that a strain could be built up, having either all rods, as in the all-rod type of J. subguttata, or all **V**'s, as in J. unicolor. I also believe that breaks in the **V**'s may occur at any time and that varying degrees of this tendency to break might exist, ranging from the condition in those compound **V**'s where no break seems to occur, such as we find true of all species of the genus Chorthippus (Stenobothrus), to that where only occasional linkage occurs, as in Chortophaga (McClung '14), or merely irregular association, as in the members of the 14 and 16 pairs in two specimens of Jamaicana flava (compare A, nos. 14 and 16, in Woolsey '15, figs. 1–4 with 5–8). From a comparison of such **V**'s, I think it reasonable to suppose that races might be built up in a species with **V**'s of any grade in respect to this tendency to break. Such, again, might form a basis for the varying degrees of crossovers Morgan obtained in coupling factors.

6. *The chromosomes: a mechanism furnishing a basis for variation, heredity and evolution?*

The observations presented in this paper seem to be but additional links in the chain of evidence going to show that in

the chromosomes we have structures sufficient in many respects to serve as a morphological basis for the phenomena of heredity, variation and evolution.

In the first place there is a number which may be considered fundamental and constant, not only for all the cells of an individual but likewise of the individuals of a species, the species of a genus, the genera of a subfamily and, if they are sufficiently closely related, even the subfamilies of a family, as may be seen in the Truxalinae, Oedipodinae, and Acridiinae, three of the four subfamilies of the Acrididae or better in the subfamilies of the Tettigidae. In the family Tettigidae, with the exception of three specimens,[5] I have found this number to be 13 (♂) and 14 (♀) for the genera I have so far examined: Choriphyllum (one species) Nomotettix (one species), Acridium (four species), Parattettix (two species), and Tettigidea (two species)

This number I found in all cells, both germ and somatic; in the testis, spermatogonia and first spermatocytes, in the hypodermis, the integument, the proctodeum, the mid-intestine, 'fat body,' muscle tissue, the follicular walls of the gonads (♂ and ♀). In the 'fat body' two cells were seen which showed instead of the usual number (13) the double number (26). This, however, may be the result of failure of the cytoplasm to divide. I have no hesitation therefore in saying that the numbers fundamental for the family Tettigidae are 13 (♂) and 14 (♀), i.e., six pairs of autosomes plus the sex chromosomes.

In the three subfamilies of the Acrididae (Truxalinae, Oedipodinae, Acridinae), over forty genera (McClung '14) so far examined have been found to possess with three exceptions twenty-three chromosomes. The exceptions are in the genus Chorthippus (Stenobothrus), where in five species seventeen chromosomes have been reported, and in material from an unidentified specimen described by McClung ('14, figs. 59–76) as having twenty-one. My work upon Chorthippus curtipennis

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[5] The three exceptions were one individual (♂) in which there occurred a deficient supernumerary sex chromosome, and two others (♂ and ♀) in which there seemed to be the one-and-one-half equivalent of supernumerary no. 1. chromosome attached to a normal no. 1 (figs. 136 to 147 and my Studies III).

shows that probably twelve of the twenty-three chromosomes have fused to form the six **V**'s which are always present. If we admit that this may have occurred, the number continues to be twenty-three for this as for other genera. The case described by McClung seems to be an exception I cannot explain. A third exception is that described by Montgomery in 1906 in Syrbula acuticornis, where the number was reported as varying from twenty to twenty-four. This, however, may have been a mistake, since I have shown in the present paper that this species may have twenty-three. For these three subfamilies of the Acrididae, the Truxalinae, Oedipodinae and Acridiinae, the fundamental number therefore seems to be twenty-three.

The phenomenon of linking as shown in the compound **V**-chromosomes of Chorthippus leads us to suspect that fundamental numbers will probably be found for the subfamilies of the Gryllidae and the Locustidae also, where **V**'s are known frequently to be present. A start in this direction has been made for the Locustidae, in the genus Jamaicana, as described by Woolsey ('15) and in this paper. Similar conditions have been found by Metz ('14) for the Diptera in the species of Drosophila. It is to be expected, I believe, that a great deal of the variation in chromosome numbers among nearly related species may be ascribed to this cause.

As the number of the chromosomes is, within certain limits, constant, so too are their sizes. It seems that no group of species or of genera so far studied presents such unmistakable evidence in constancy of size gradations as do the genera of the Tettigidae. There may readily be seen in all cells of the body, both somatic and germinal, a series of relative sizes constant for the six pairs of autosomes and for the single ($\male$) or paired ($\female$) sex chromosomes. These size gradations are constant for the individuals of a species and, with slight variation, for the species of a genus; likewise, with somewhat greater variations, for the genera of a family. Of the autosomes there may be recognized two very small pairs, two intermediate ones and two extremely large ones. The two pairs within each of these three groups may in turn be distinguished by slight difference

in size. These size gradations are so dependable that one may follow without difficulty any chromosome pair he chooses through the cells of all of the species and genera I have so far studied. If I see the whole series or, in some cases, even a part of it, I can recognize a no. 6 or a no. 4, for example, as such. An idea of the degree of constancy may be obtained by following any chromosome through the figures from 23 to 147. In Protenor belfragii Wilson ('05) was able to recognize in all cells only the two largest and the two smallest pairs of chromosomes. In the Tettigidae it is possible to recognize every one of the series of six autosome pairs as well as the sex pair.

In the Acrididae[3] again size gradations are recognizable with a considerable precision, as may be seen in the species of Stenobothrus (Meek, '12), Syrbula (Robertson, '08), Melanoplus (Nowlin, '08) and numerous other genera. There may always be readily recognized three small pairs and three large ones with a graded series of intermediates. The sex chromosome ranks from eighth to tenth in the series of twelve pairs. With the exception of the three largest and three smallest pairs and the sex chromosome, it is impossible to trace very definitely individual chromosomes through these three subfamilies unless the chromosomes happen to be in some way linked together as in Chorthippus.

Such constant size gradations recognizable in both germ and somatic cells is evidence against the theory of King ('08) and Haecker ('11) that size gradations are the result of unequally rapid development or growth. The occurrence of these size gradations in somatic cells is also an answer to Haecker's ('11) criticism that such gradations have not been found outside of germ cells.

As in number and in size, so in their behavior, the members of a series of chromosomes are constant to a greater or less degree. In the Tettigidae all spermatogonial, somatic and second-spermatocyte chromosomes are straight rods, pointed at the proximal, and rounded at the distal end. In the prophase and metaphase no rings like those in the Acrididae (Syrbula, fig. 157, no. 10) have thus far been found. There seems to be a

predominating tendency for the four-strand tetrads to divide throughout a large part of their length along the plane of the primary split (*I*), the division beginning at the proximal end, and the halves remaining attached at the distal ends. There results the dumb-bell shaped rods (figs. 29–31, 73–77, etc.) of the late prophase and the metaphase. Frequently in the longer chromosomes of Tettigidea there is a tendency for a tetrad simultaneously to split also from the distal end along the plane of the second division, so that there is formed a cross with unequal arms (figs. 94–99, 112–114), the transverse arms being short, even mere knobs. For the Tettigidae the characteristic form of prophase tetrads in tetrads formed from shorter chromosome pairs is the dumb-bell rod, and in tetrads from longer chromosomes it is the cross with very short transverse arms.

In the species of the subfamilies having twenty-three chromosomes, there is a prevailing tendency in the pure straight-rod type for the longer and intermediate chromosome tetrads to split widely along the plane of the secondary division. If this begins at the distal end and extends almost to the proximal end the result is the **V**-tetrad (fig. 157, no. 10, and Robertson, '08, plate 21, chromosomes 4, 6, 8); if it does not begin at the distal end, but at a point just proximal to it, and extends as before nearly to the proximal end, there results a horizontal ring (11 in fig. 157 and Robertson, '08, plate 21, chromosomes 7, 9–12); or, in the case of the intermediate chromosome pairs, if the widening of the primary split, beginning at the proximal end, is equal to that of the secondary beginning at the distal end, a cross tetrad results. The three smallest tetrads usually form dumb-bell shaped tetrads, as in Tettigidae (Robertson, '08, plate 21, chromosomes 1–3). Chromosomes of the ring-V-tetrad-, and cross-type are characteristic for these subfamilies, while those of the dumb-bell-, rod-, and unequal-armed cross-type are characteristic for the Tettigidae. The behavior of tetrads in producing these forms is not such a constant characteristic, however, as is number or size. This may be seen in my chromosome table ('08, plate 21). While there is a tendency to produce tetrads of a certain form, rings for instance, in no. 10,

that tetrad does not always do so, as may be seen in cells 5 and 6 of this table.

While the behavior of chromosomes in forming tetrads is not always constant, as Moore ('06) and Baumgartner ('04) believed, but only tends that way; their behavior, on the contrary, in either forming or refusing to form certain associations resulting in compound chromosomes seems to be constant in some cases (Jamaicana subguttata), only for the individual, in others (Drosophila ampelophila) for the species or in still others (Chorthippus, Syrbula, Hesperotettix, Mermiria) for the genus. In Chorthippus curtipennis, for example, there are present in all individuals of the species so far examined three pairs of V's. These pairs of V's may be distinguished and recognized by the relative lengths of the two arms, which differ enough to enable one to recognize the V's individually, not only in all cells of this species, but—judging from figure 6 of Meek ('12b) and those of Gerard and of Davis—in other species of the genus as well. The lengths of these arms in Chorthippus are almost identical with the relative lengths of the nos. 5, 7, 8, 9, 10, and 11 chromosomes in Syrbula, a nearly related genus, in which no V's are formed. The behavior of these pairs, so far as our knowledge goes, is therefore constant within one genus (Chorthippus) in forming V's, and in another genus (Syrbula) in not forming V's. The same may be said of the sex chromosome and the particular autosome with which it forms a compound (V-chromosome) in the species of Hesperotettix, when compared with their behavior in failing to do so in the majority of other genera. Likewise in Mermiria, and especially in the four large pairs of rod chromosomes in Drosophila, as Metz has shown, where in one type of species they remain independent rods, in another type two pairs of the rods unite to form a pair of V's, while in a third type the four pairs of rods unite to form two pairs of V's.

Individual, or at least genetic, continuity and specificity is shown in chromosomes that are traceable. In the Tettigidae the fact that we may trace and recognize any particular pair of autosomes from the no. 1's to the no. 6's, as well as the sex

chromosome, in any cell of the body, and not only for the cells of an individual or a species but even in the different species of a genus and in different genera and subfamilies (though they may have suffered considerable modification), is evidence to my mind that in these structures we are dealing with at least genetically continuous bodies. The same may be said in regard to the V-compound chromosomes of Chorthippus. Here we evidently have six pairs of rod chromosomes, so linking as to form three pairs of V's which may be recognized individually in all cells; for spermatogonia in the diploid relation; for first spermatocytes in the pairing relation in all stages of synapsis from stage 'i' on to and through the metaphases, and (in disjunction) in the anaphases; and again in the haploid relation in the second spermatocytes and spermatids. This habit of association or linking in the case of these six particular pairs of autosomes is the individually continuous relation which is surprising. In the majority of genera it does not occur, at least not for all six of these pairs of autosomes, but here in a number of species occurring widely distributed in both Europe and America, which systematists have, upon the basis of similarity in external body characters, grouped into a single genus, this habit of association between members of these six particular pairs is constant. This signifies to my mind that there must be more than a genetic continuity; there must be an individual continuity of each specific V-chromosome through the spireme stage from cell division to cell division, for it does not seem likely that the connecting substance in the achromatic bridge between two particular rod chromosomes in the case of each V would be dissolved in a telophase only to be formed again at the next prophase.

If the interlocking which I have figured and described (fig. 163) and the Schreiners ('06b, figs. 24, 25) have likewise illustrated is to be taken into consideration, then we have again very definite evidence not of genetic but of individual continuity through the stages of the first spermatocyte nucleus, where most doubt prevails as to the persistence of the continuity of the chromatin thread.

Again the one-V-type individual of Jamaicana has an important bearing on the subject of individual continuity. Here in the same animal one member of each of the pairs 14 and 16 exists as a separate rod in every cell, while the mates of these exist in the same cells linked together. Whether the chromosome in the ancestral species was a **V** or two rods, I cannot say. If the ancestral chromosome was a **V**, then a break has occurred at some time and this break has been handed down generation after generation. On the other hand if a fusion has occurred at some time, then the fusion condition has been handed down. Whichever hypothesis we adopt, this much seems certain, that the thread in the case of the **V** retains its identity from cell to cell and likewise in the case of the rods. If we argue for a fusion of the rods to form the single **V** at each prophase, how can there exist in the same cell homologous rods which do not form **V**'s? This to me is an argument against the 'manouvre hypothesis' of Fick, viz., that chromosomes represent tactical formations produced anew in the cell, and the somewhat similar hypotheses advanced by Meves, Giglio-Tos and Granata.

It seems to me that further strong arguments in favor of the individual continuity of the chromosome thread are the deficient no. 4 of Tettigidea (figs. 104, 110, 115, 119, 120, 122 and Study III) and the no. 1's of Acridium (figs. 136–147, and Study III). Here we have in these animals every cell in the body, both germ and somatic, showing in the one case the defective no. 4 chromosome, in the other case the abnormally long no. 1. That these chromosomes were probably present at the fertilization of the egg, is shown in the first case by their presence in every cell of the body and in the second case by their being distributed as units to half the second spermatocytes. This is also reinforced by the fact that the same abnormal long chromosome has in all probability been found in both male and female animals. That these chromosomes are abnormal, I think no one who reads the description of them will doubt. Now, in the case of the defective no. 4, all cells in division showed it to be of constant relative size. The same may be said of the long no. 1 of Acridium; not only is it true for all cells of one

animal, but likewise for those of another individual, a female taken at the same time and from the same spot as the male. Both chromosomes were probably handed down from preceding generations. The behavior of this chromosome, it seems, to me, argues for the persistence of individual continuity in the chromatin thread from cell to cell. Otherwise, how could the constancy of size be maintained, especially in the case of the defective chromosome?

These again are arguments opposed to the views of Haecker ('11) and King ('08) that the differing sizes of the chromosomes are due to unequal growth. If their theory be correct, why these permanent abnormalities in size?

It seems to me that these abnormal chromosomes are even more conclusive arguments in favor of continuity than the behavior of the supernumerary chromosomes, for in the latter we have more or less independent bodies in the cell—in the former, abnormal bodies linked up in one case with a chromosome which we can readily recognize in the cell. We also have the advantage in Tettigidae that we may there recognize all of the chromosomes individually.

Specificity of chromosomes is so closely bound up with individuality and genetic continuity that it is not necessary to discuss it at length. Montgomery has pointed out that specificity of function is at least implied in the permanent size differences in so far as amount of chromatin is concerned, a large chromosome having more with which to maintain a function than a small chromosome. Another case of specificity of chromosomes is shown in the tendency of one pair (no. 4's) to precede the other autosomes in condensation, and also that of at least one member of this pair to associate with the sex chromosome in the first spermatocytes of Syrbula and Chorthippus (figs. 149, 150, 155, 163–165, 168–170, 171–174, 178–180). Another case that might be cited is the occurrence of the V and its rod-mates in Jamaicana unicolor (figs. 196–199) within the same cell. This might be cited as exhibiting not only a difference from the rest of the chromosomes in forming V's, but likewise a difference in speci-

ficity between members of the same pairs. The same may be said of the no. 4's in the deficient no. 4 tetrad.

And now it seems to me that in the foregoing data on constancy in number, size and behavior, and in the evidence for genetic—and in some cases individual—continuity of the chromosomes, we have, especially in the Tettigidae, proof of the presence in the germ cells of a structure sufficiently stable and continuous to furnish a physical basis for heredity. Again, this structure, while sufficiently stable to account for heredity, the relationship of species, genera, etc., has evidently been plastic enough to allow of variation in the past sufficient to account for what we find characteristic of at least two subfamilies of the Tettigidae, the Batrachidinae and Tettiginae, and of the genera within these, when compared with each other, and of the respective species of the genera, in so far as we are able to see.

The family Tettigidae itself in its thirteen-chromosome trait is distinctly and clearly marked off from the subfamilies Truxalinae, Oedipodinae and Acridinae of the Acrididae in their twenty-three chromosome trait. This degree of difference, as I have already pointed out, is paralleled in the somatic characters, both internal and external. The degree of difference in relative length of chromosomes between the genus Tettigidea, of the subfamily Batrachidinae, and the two genera Paratettix and Acridium of the Tettiginae is greater than is the difference between the genera Paratettix and Acridium themselves. This may be seen in the tables (I–XX). Among the species of Acridium the same phenomenon may be seen, though to a much less degree. As we descend to the species, the differences between nearly related ones are so little that it is practically impossible to detect them.

The variations I have described are found existing today, permanent for a number of organisms which systematists have grouped into families, subfamilies, genera, and in some cases species. Whether any one of these three genera of the family Tettigidae represents the more ancestral genus of the family, I am not ready to say. This may be determined later. Suffice it to say that from the evidence displayed in the series of chromo-

somes, descent by variation from a common ancestral series of chromosomes may certainly be inferred, and the degrees of variation shown in these chromosomes is paralleled by the degrees of variation exhibited by somatic structures, which systematists have made use of in showing the relationship of species, genera, subfamilies, etc.

Variations which have occurred in the Acrididae and which seem to be of a permanent nature are the fusion of non-homologous autosomes to form compound chromosomes as in the species of the genus Chorthippus. That these are variations is to be inferred from the fact that about nine-tenths of the genera of the twenty-three-chromosome grasshoppers do not possess these V's, while in one-tenth, and especially this one genus Chorthippus, the species so far described (5) all show this variation. It is interesting to note that it occurs in both the American and the European species of the genus. In Hesperotettix McClung ('05) has described a somewhat similar occurrence, though here the sex chromosome is concerned. The variation is constant for all species of the genus that have been examined. Likewise in the genus Mermiria McClung ('05, '14) has described associations similar to Hesperotettix but in which *two* autosomes seem to be linked up permanently with the sex autosome.

In Jamaicana we have a third case of such chromosome variation, which, however, is peculiar in that two variations, each apparently permanent, exist side by side within the same animal, as the compound **V** paired with its rod-mates seems to show. The associated rods evidently remain together through spermatogonial and first and second spermatocyte generations, and are found even in somatic cells, while their rod-mates remain separate. This case is of great importance for it shows that chromosome variations characteristic of two classes of individual animals may exist side by side within the same cell, apparently independent of each other, conditions we should expect to find in the cells of hybrid animals.

These, it seems to me, are permanent variations of a fundamental type; but in addition to these, I have described in the

present paper and in Studies III a class of variations that seem
to be of an abnormal type, which, as I have pointed out in Study
III, are also probably of a permanent nature. One of these
(figs. 136–147) is probably to be placed in the class of 'supernu-
merary chromosomes.' Up to this time the supernumerary
chromosomes described have been either extra sex chromosomes
or chromosomes in some way partaking of the properties of sex
chromosomes in that they are usually condensed. Here we prob-
ably have in the $1\frac{1}{2}$-valent portion of the large abnormal chromo-
some 1 the remains of supernumerary no. 1's strung out in a
row (compound-supernumerary). Just what relation to varia-
tion they may have, it is difficult to imagine. A more impor-
tant representative of this abnormal class for matters of varia-
tion, it seems, is the deficient no. 4, whose measurement (Studies
III) shows it clearly to be about four-fifths the length of its mate,
which has the size normal for the halves of the pair in the species.
In such a chromosome we have the basis for the dropping of
unit factors from the germ plasm. This, I think, might explain
those characters which are evidently due to the lack of some-
thing, such as pigment or its producer in albino animals, and
come under the class of 'loss' unit characters. But such sorts
of variations (mutation) are not, it would appear, to be classed
with variations which mark off species from each other, such for
instance as distinguish Acridium granulatus from A. obscurus.
They are, instead, to be classed as minor variations occurring
within a species and possibly of little use in the production of
new species. It is not possible to consider them normal, for it
might be a question whether a race having such could be per-
manent until at least some additional compensating variation
occurred to supply what is lacking. Possibly there may be
some connection in this respect between compound-supernumer-
ary autosomes (1) of one pair and the deficient autosomes of an-
other pair.

Our conclusion is that in the chromosomes of the germ cells
variations *have occurred*, as shown by taxonomic relations, and
may again occur, as shown by deficient and compound-super-

numary chromosomes, and that according to all indications they
are *permanent*.

The fact that three genera of Tettigidae show the same general
plan of chromosome structure leads us to suppose that within
each of these three genera we might look for the same general
sort of variations, such as could be dealt with by the Mendelian
law. Nabours ('14), in his work upon the inheritance of color
markings in Paratettix, has shown that their behavior conforms
to this law in one of these genera. He finds nine pure strains
showing distinct color patterns, and in addition to this eighteen
heterozygous patterns from combinations of these pure strains.
It is a striking fact that in collecting specimens of three other
genera, Acridium, Nomotettix and Tettigidea, I have found many
of the same markings. All of these genera have individuals
showing the white spot on the large femora which Nabours
has described in his Paratettix punctofemorata (fig. D, plate
VI). The spot varies slightly in shape and orientation, but
nevertheless it is a white spot on the same region of the rear leg.
Likewise I have found all four genera to have individuals with
a white band across the thorax, such as he has described in his
leucothorax variety (fig. C, plate XI) and as Hancock ('02) has
shown for Acridium (Tettix) in the fourth specimen of his figure
1, page 9. I have also found in all four genera the leuconotus
variety shown by Nabours at B, plate VI, and by Hancock in
figure 1, specimen 6. The variety with a median stripe of the
shape of that shown at E (Nabour's fig. VI), but white instead
of yellow, I have found in all four genera. Hancock has also
pictured this variety for Acridium in his figure 1. The gray
variety carrying small patterns of black, like A of Nabours and
the first specimen of figure 1 in Hancock, is probably the most
common of all patterns in all of the genera.

The genera Nomotettix, Acridium and Paratettix belong to
the same subfamily (Tettiginae), while Tettigidea belongs to an
entirely distinct subfamily, the Batrachidinae. The germ-cell
structure shows Tettigidea to be farther removed from the other
three genera than they are from one another. Basing my opinion
upon a knowledge of germ cells alone, I should expect to find in

this genus the *same* minor variation (such as Nabours has worked out), but *considerably modified* from the condition found in the three more closely related genera. This seems to be the case in regard to at least the 'punctofemorata,' 'leuconotus' and 'lineatus' characters. Such characters are present in Tettigidea, but apparently widely different from what they are in any of the other three genera.

It appears to me that in the varying behavior of the chromosomes among the genera of this subfamily we have a splendid opportunity, by repeating on other species and genera the work of Nabours, to find out something of the workings of the chromosomes in respect at least to minor, non-vital variations, such as these seem to be.

The chromosome studies which I here present were begun at the University of Kansas in 1908 under the direction of Prof. C. E. McClung. A part of my material was collected at Lawrence, Kans., and also in the course of zoological collecting trips on which he was kind enough to send me to Arizona, Texas, and Puget Sound, Washington. The studies were continued at Harvard University from October, 1909, to June, 1912 under the direction of Prof. E. L. Mark. I am indebted to both of these men, to Professor McClung for starting me upon the work and for his kindly interest afterward, and to Professor Mark for his helpful criticism and guidance and for the encouragement he gave me during the time when the bulk of the work was being done.

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EXPLANATION OF PLATES

All figures except those on the first three plates are reproduced at the same scale. The drawings were outlined with an Abbé camera lucida at a magnification of 3900 diameters, obtained with a Leitz 1/12 homogeneous immersion objective and a Zeiss 18 × compensating ocular with draw-tube set at 150 mm. and drawing made at the level of the base of the microscope. Having been reduced one-third in the reproduction, they now show a magnification of 2600 diameters.

ang.hum., humeral angle
ang.p., posterior angle.
apr.prc., apical process of pronotum
c., chromatoid body
cap., head
car.m., median carina
car.vrt., carina of vertex
cav., cavity of intestine
cla.cl.i., (by mistake for) cta. chi.
cl.apx., apical cell
cl.tis.co'nt., connective-tissue cell
coe.ga., gastric coecum
coln., colon
con., cone-like body
cta.chi., chitinous cuticle
cul.crc., circular ridges
cys., cyst of testis
dk., darkly stained material
dst., distal

ely., elytron
fem.a., femur of anterior pair of legs
fis., split
foll., follicles
gl.rt., rectal glands
iglv., crop
il., ilium
lob.l., lateral lobe of pronotum
lob.su'oc., supra-ocular lobe
mat.1., first maturation division
mat.2, second maturation division
ms'thx., mesothorax
mu.lg., longitudinal muscle of intestine
nll., nucleolus
occ., occiput
oes., oesophagus
par.cys., cyst wall
par.foll., follicular wall
par.nl., nuclear wall

pli.al., wing folded
pro'nt., pronotum
pro'nt.a., anterior margin of pronotum
prx., proximal
pullv., pulvillus
rt., rectum
seg.tar., segments of tarsus
sn.p., posterior, or clytral, sinus
sn.if., inferior sinus
sp'cy., cyst of first spermatocyte generation
sp'd., cyst of spermatid
sp'go^7., cyst of 7th generation spermatogonia
sp'go^8., cyst of 8th generation spermatogonia

sp'go.^{9}pr., primary spermatogonia
sp'go.scd., secondary spermatogonia
spi.t., terminal spines of tarsus
sp'zo., cyst of spermatozoa
tb.Mpg., Malpighian tubule
te., testes
tib., tibia
v., ventriculus
va. df., vas deferens
vrt., vertex
x,x., proximal ends of chromosome
x, sex chromosome
I., primary split of chromosome
II., secondary split of chromosome
1, abnormal no. 1 chromosome

PLATE 1

19 Longitudinal section of follicle of testis from Acridium granulatus. A cyst of the seventh generation secondary spermatogonia ($sp'go.$ 7) contains 64 cells; one of the eighth generation secondary spermatogonia ($sp'go.$ 8), 128 cells; cysts of the first spermatocyte generation ($sp'cy.$), 256 cells each; and cysts of spermatozoa ($sp'zo.$), 1024 cells each.

20 Longitudinal section of follicle of testis of Dissosteria carolina. After Davis ('08).

21 Testis of Acridium.

22 Testis of Dissosteira carolina. From Snodgrass's 'Anatomy of the Carolina Locust.'

19

20

21

22
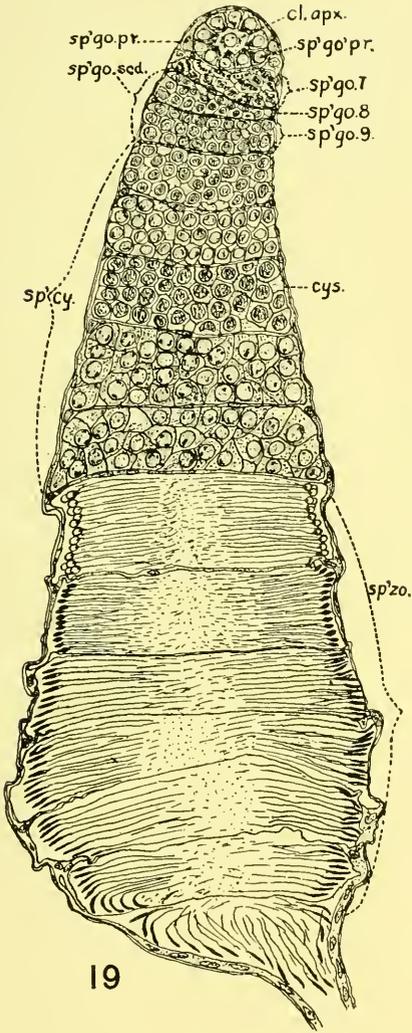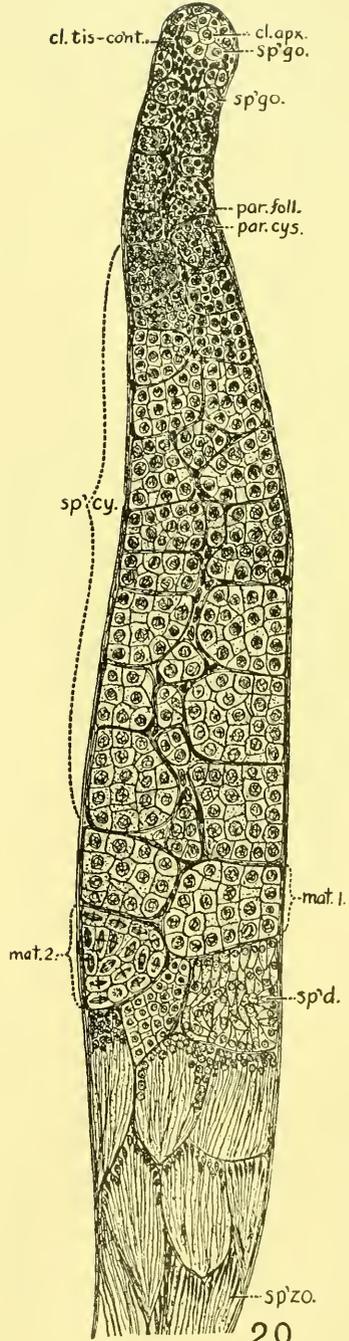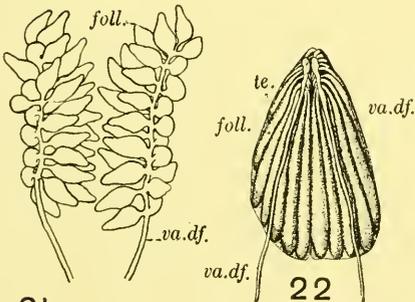
23 to 58 Acridium granulatus Scudd.

23 Secondary spermatogonium from young cyst. Only chromosomes and cell boundary shown. Ordinary chromosomes numbered according to size from 1 to 6. Sex chromosome, 2 x, ranks second in the series between ordinary chromosomes nos. 1 and 2. The number 2, preceding x, indicates this rank. It stains lightly. The deficiency of one no. 4 chromosome (4−) is shown by the minus sign (−) following the no. Sex chromosome lightly stained.

24, 25, 27, 28 Spermatogonia from various animals. Sex chromosomes ($2x$) faintly stained and 'wooly.' Deficient no. 4 in figure 27.

26a, b, c, d 'Wooly' sex chromosomes from different spermatogonial metaphases.

29 to 34 First spermatocyte divisions. Members of pairs in each case are separating from each other. The sex chromosome ($2x$) goes to one pole ahead of the others.
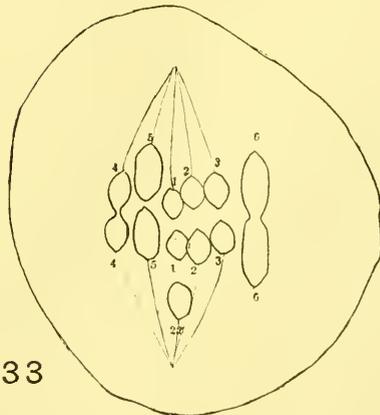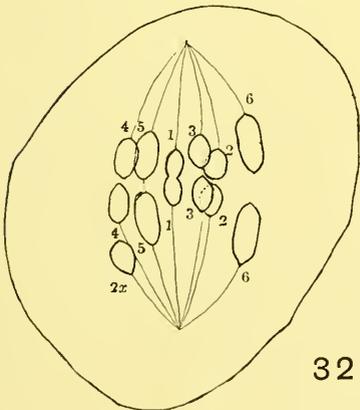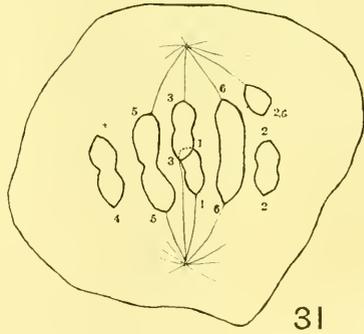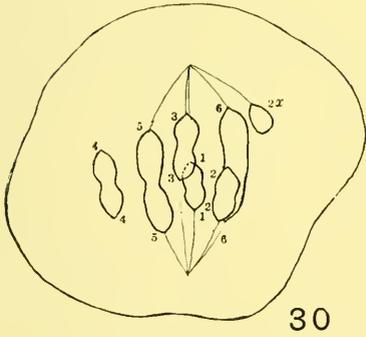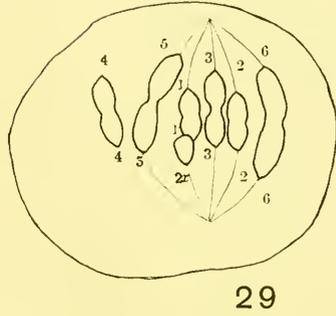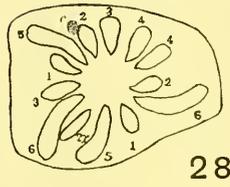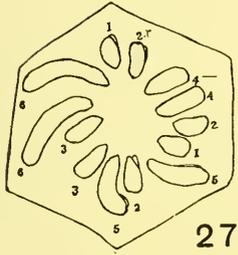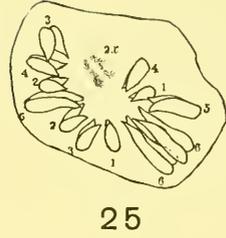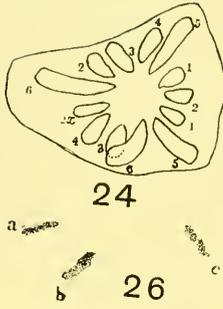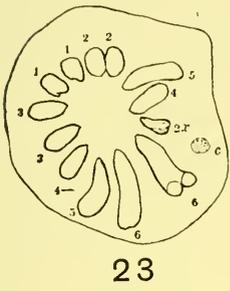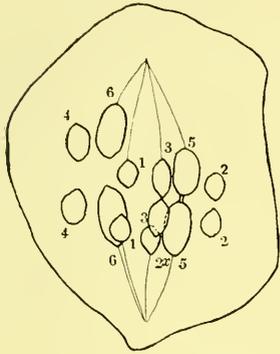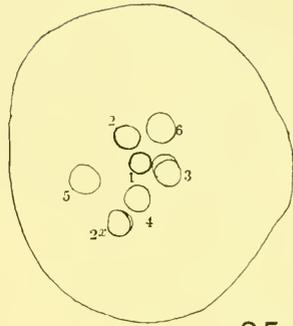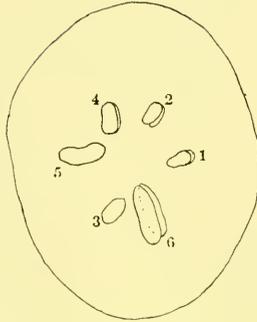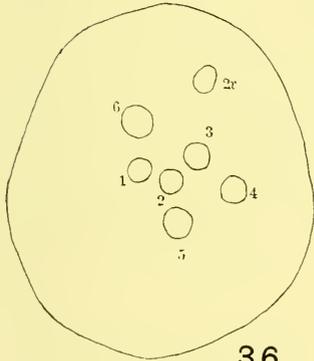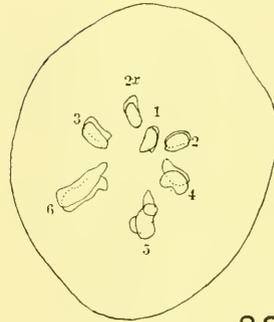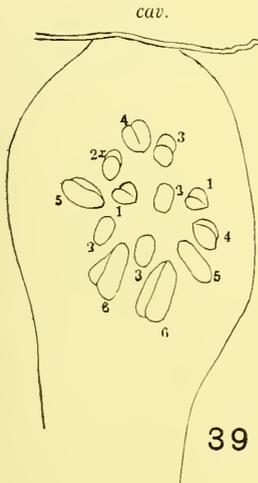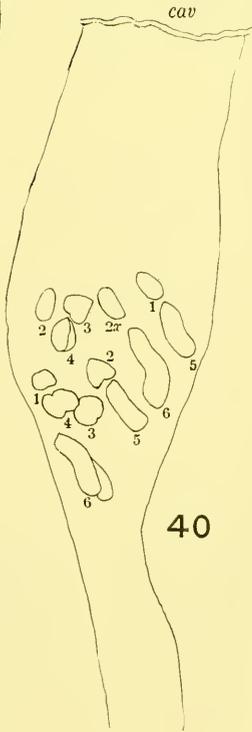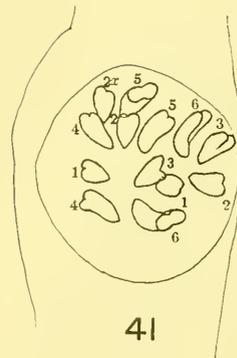
42 Proctodeal cell from same animal as figure 23. Deficient no. 4.

43 Proctodeal cell.

44 From the hypodermis. Contains many pigment granules.

45 Hypodermis.

46 Giant cell from 'fat body.' Twenty-six chromosomes.

47 Hypodermis cell.

48a and b Proctodeal or connective-tissue cell in contact with muscular coat of intestine.

49 From the 'fat body.'

50a and b From hypodermis.

51 to 54 From female. Ovarian follicle cells. Fourteen chromosomes in each. The two sex chromosomes $(2x)$ may be recognized by their looser, more faintly stained character. A precocious synapsis of the no. 1's in figure 51.

291
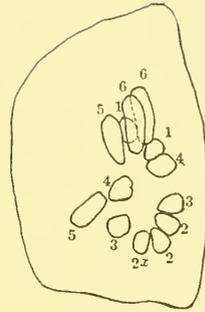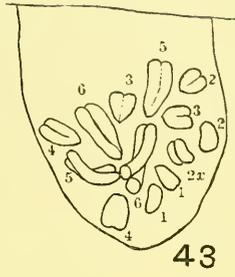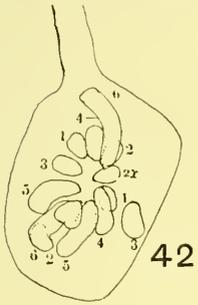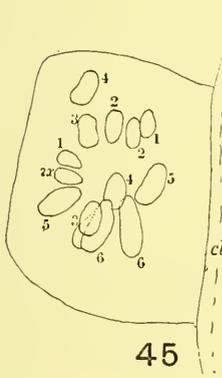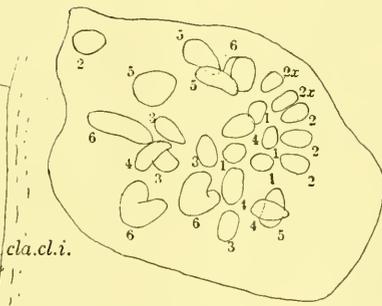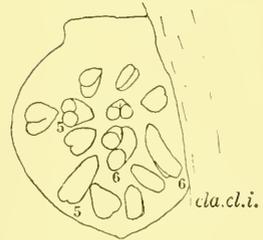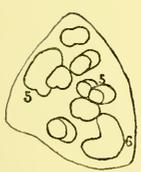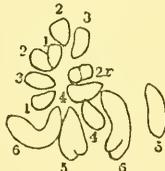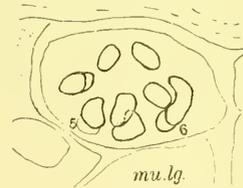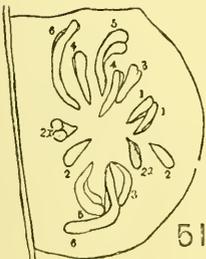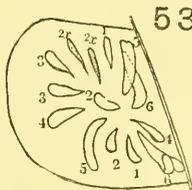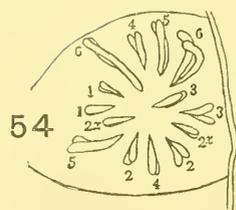
55 to 58 Acridium granulatus. Ovarian follicle cells, continued. Sex chromosomes 'woolly' (fig. 58) and loose in much decolorized cells.

59 to 64 Acridium incurvatus Hanc.

59, 60 From wall of follicle of testis.

61 to 64 First spermatocytes, late prophase. One small pair (no. 2) is stubby, the other is long and slender. In figure 61 the no. $1x$ has been drawn out of its normal position. In figure 63 nos. 4, 5, and 2 have been treated similarly. $1x$ is seen in end view in figure 63.

65, 66 Acridium ornatus Harris. Prophase of first spermatocyte.

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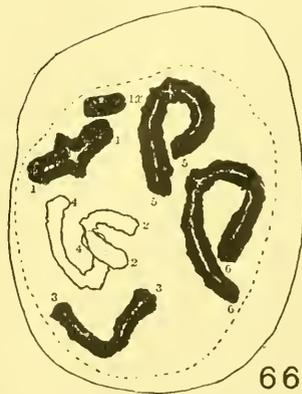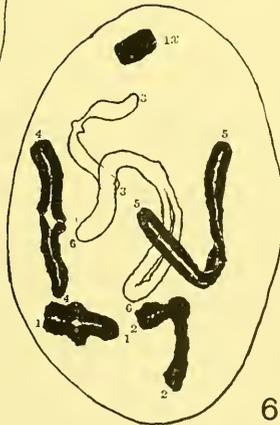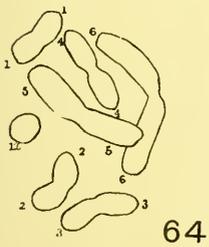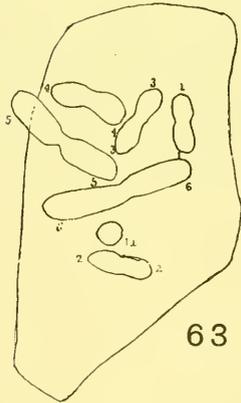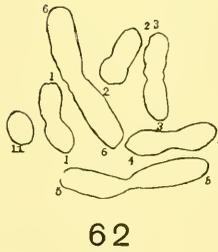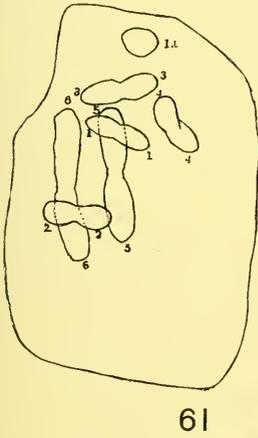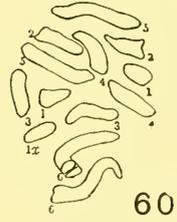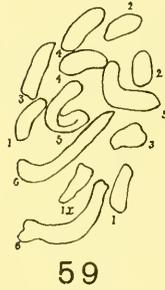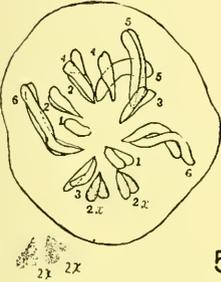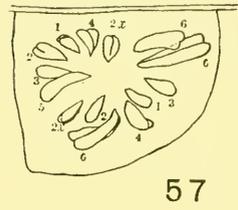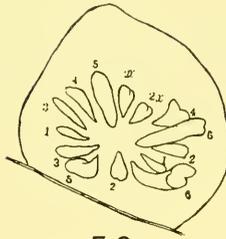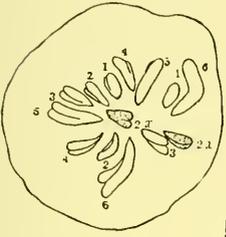
67 to 77   Acridium obscurus Hanc.

67, 68 Spermatogonial cells. Sex chromosome faintly stained. Autosomes black. Chromatoid body (*c*) not always present.

69 to 71   Prophase of first spermatocyte.   Homologous chromosomes in synapsis by distal ends only. *con.*, probably a fragment of a chromosome.

72 to 75   First spermatocytes in division. *con.*, split in figures 72 and 73, possibly attempting to divide.

WM. REES B. ROBERTSON
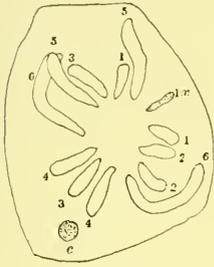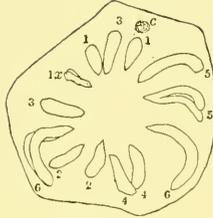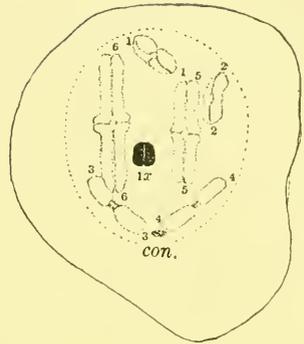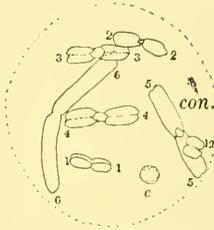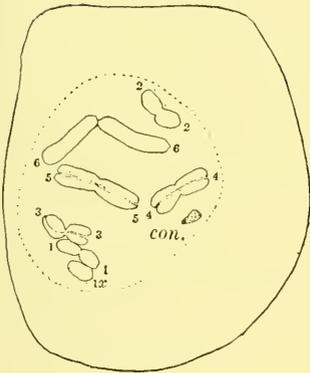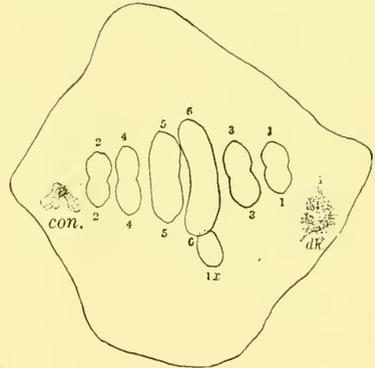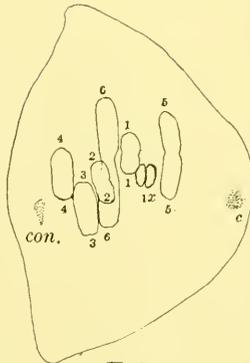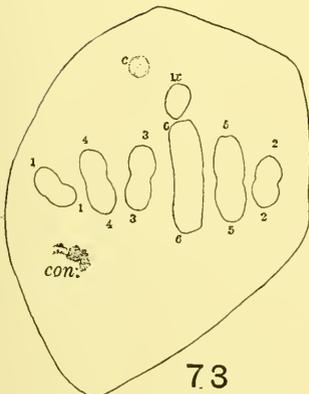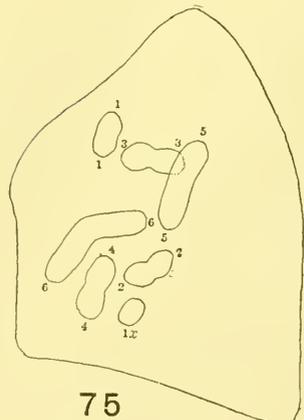
76 and 77   A.   obscurus.   Late stage of first spermatocytes.

78 to 84   Paratettix cucculatus Morse.

78 to 82   Spermatogonial cells.   Sex chromosome ($3x$) is 'wooly,' as usual. In figure 81 the 6's and one no. 5 are foreshortened.   Figure 82 is from a cyst of dividing cells which contained an apical cell in the center.

83   First spermatocyte division.
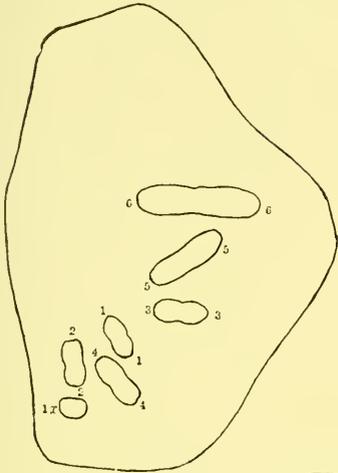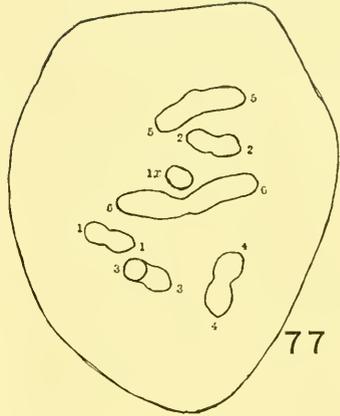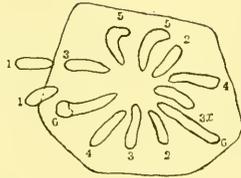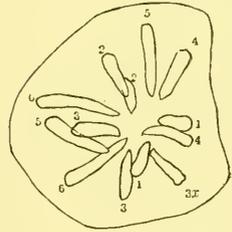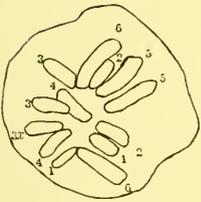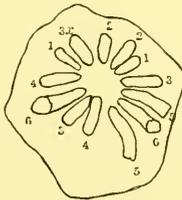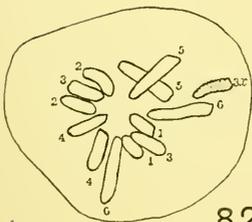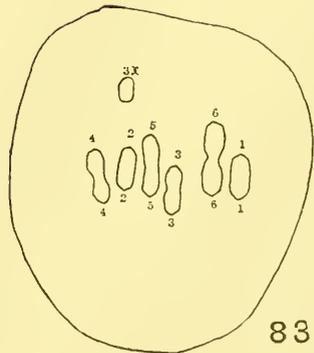
PLATE 10

84 P. cucculatus. First spermatocyte.

85 to 89 Paratettix texanus Hanc. First spermatocyte division.

90 P. texanus. Second spermatocyte metaphase showing relative size of all chromosomes.

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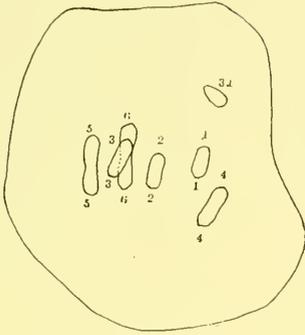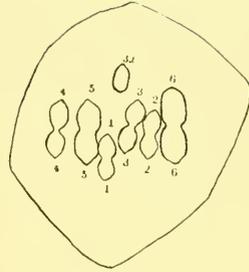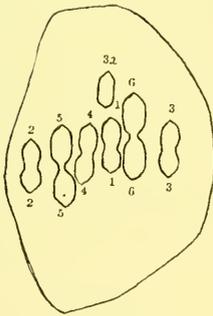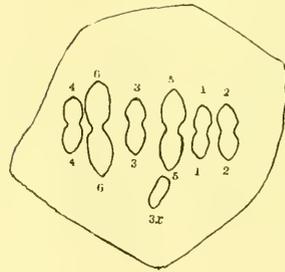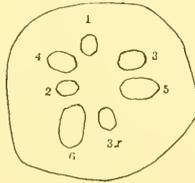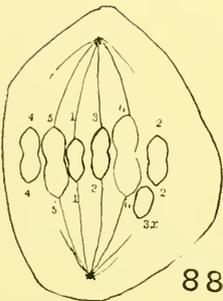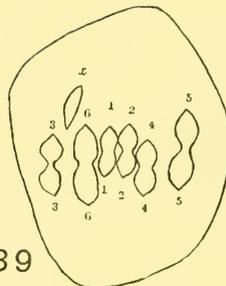
91 to 100   Tettigidea parvipennis pennata Morse.

91   Spermatogonium, 13 chromosomes. Sex chromosome ranks fifth in the series, being between the fourth and fifth autosomes in size.

92   Spermatogonium, 13 chromosomes.

93 to 98   Late prophases of first spermatocyte. Chromosomes in some cases illustrated out of their natural position to provide more room.

99   First spermatocyte division. The no. 4 pair is lacking, lost in sectioning the cell.
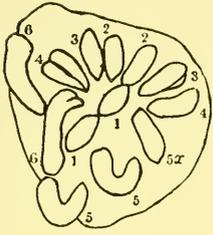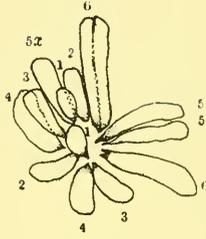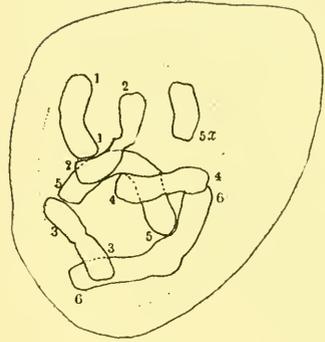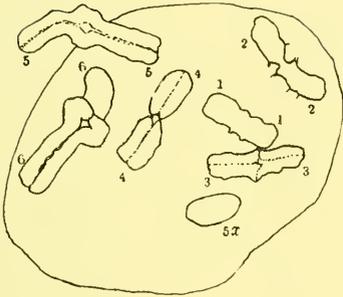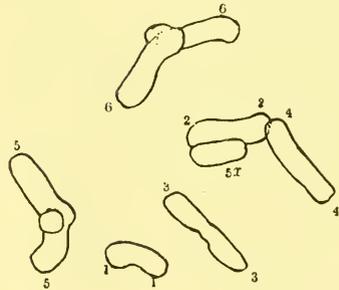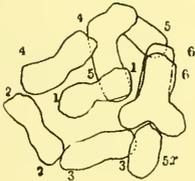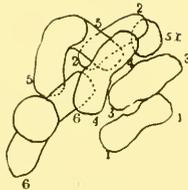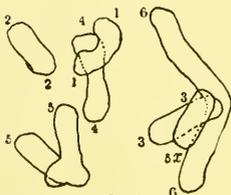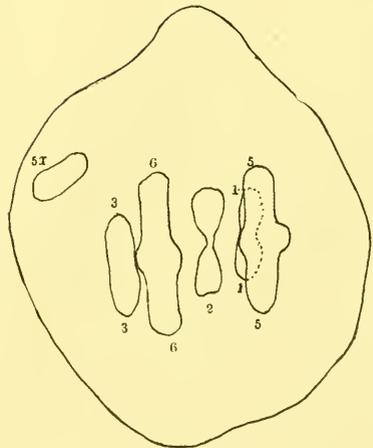
100 T. parvipennis pennata. Oogonium, 14 chromosomes.

101 to 127 Tettigidea parvipennis Morse.

101 to 103 Cells from the ovarian follicle. Fourteen chromosomes; two no. 5x's in each cell. Chromosomes show their split.

104, 105, and 107 Spermatogonial cells all from same animal but from different cysts of testis; 13 chromosomes. One no. 4 (4−) is deficient in length. Chromosomes show their split.

106 5's and 6's from various cells, similar to those of figure 104, showing the knobs on the ends of the chromosomes.

108 to 110 From dividing cells in the 'fat body' surrounding the testis of same animal from which figures 105 to 107 came. A deficient no. 4 chromosome (4−) present in each case.

108a and b are the parts of one cell from two sections.

110 is in anaphase.
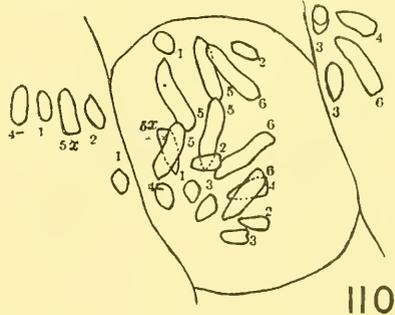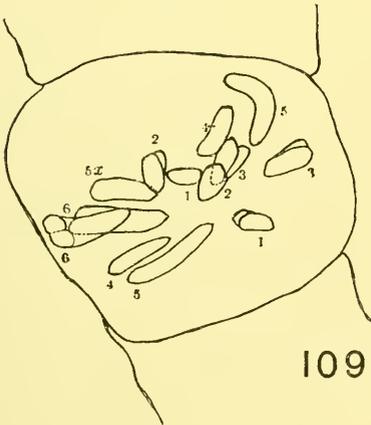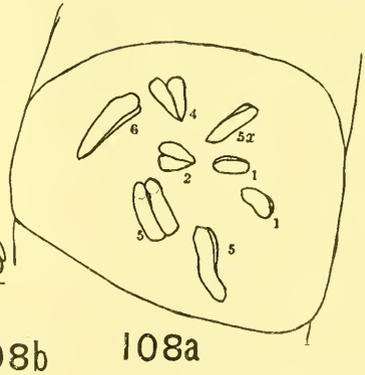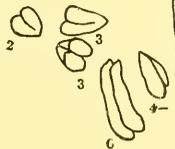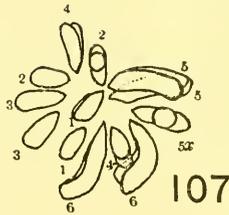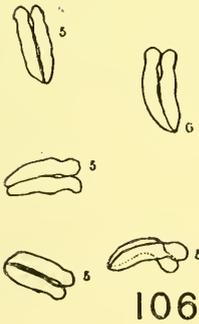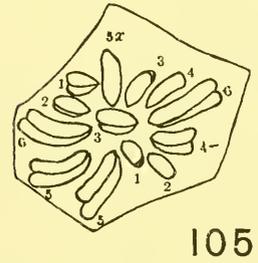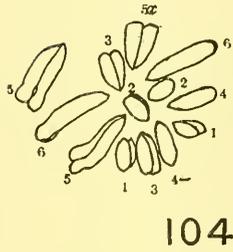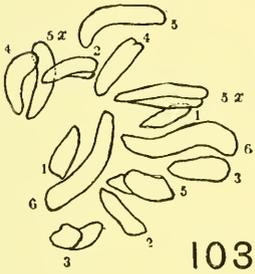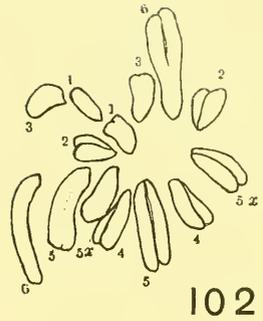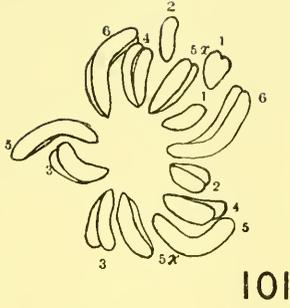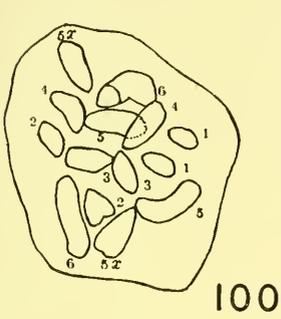
PLATE 13

(Tettigidea parvipennis)

111 to 118 First spermatocyte divisions. Full number of chromosomes in each cell. Figures 115, 118, containing the deficient tetrad no. 4, are from same animal as figures 104, 105, 107 to 110. Note inequality of the no. 4's.
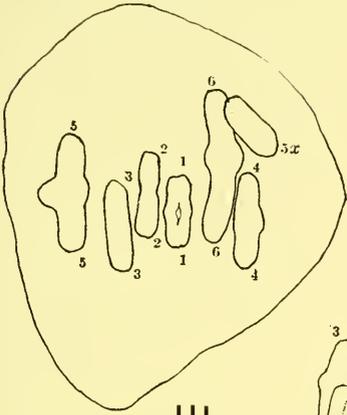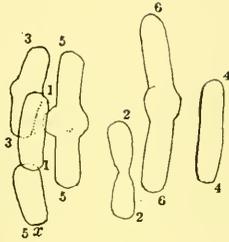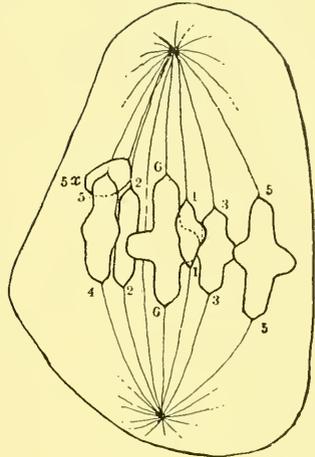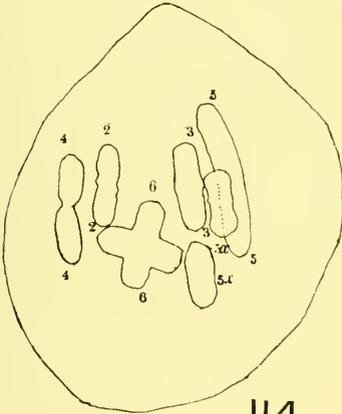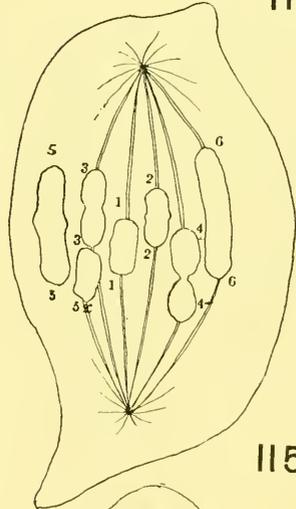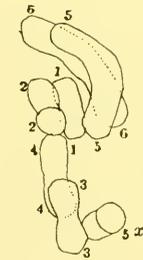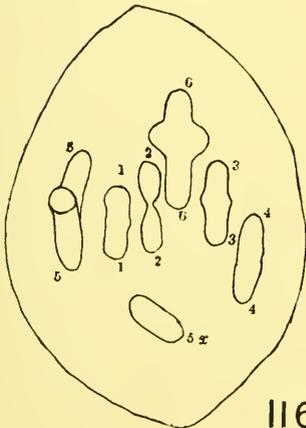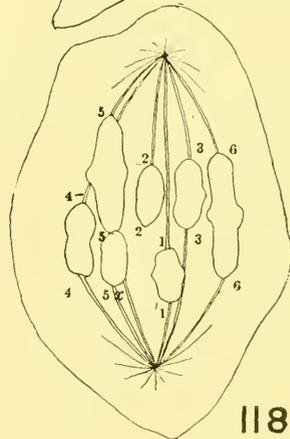
119, 120, 122   First spermatocytes from same animal as figures 104, etc.

120   Three of the unequal no. 4 tetrads compared.   Note uniformity in sizes of deficient and normal no. 4's; also irregularity in position of points of attachment.

121   First spermatocyte, showing relative sizes of chromosomes except no. 5, which is lacking, lost in sectioning.

122   Anaphase of first spermatocyte.

123   Late anaphase showing lagging sex chromosome.

124, 127   Second spermatocyte metaphases containing the sex chromosomes. Figure 124 is a mate to figure 125.

125, 126   Second spermatocytes lacking sex chromosome.   126 is a mate to figure 127.
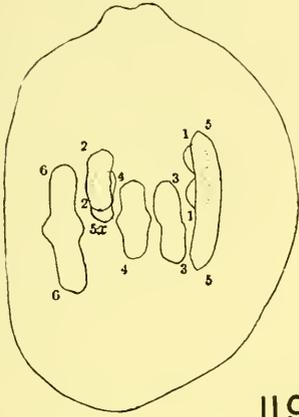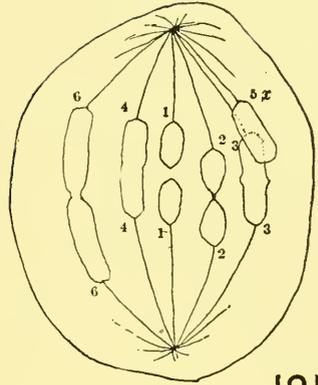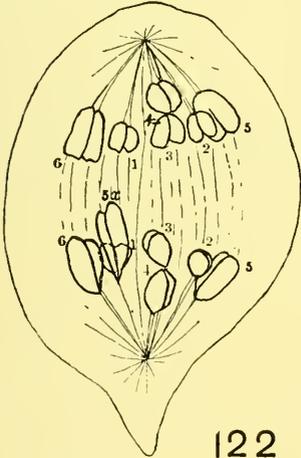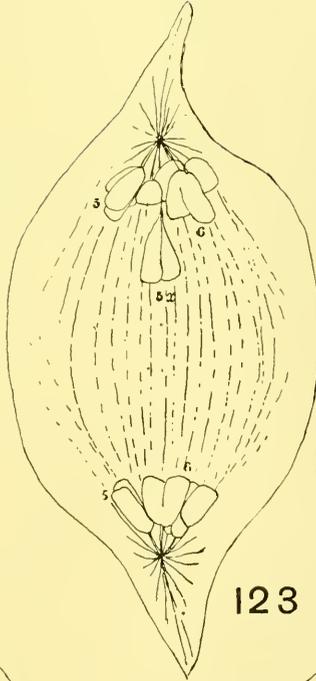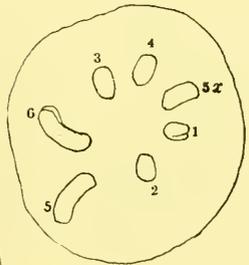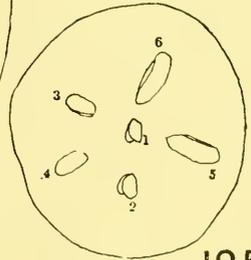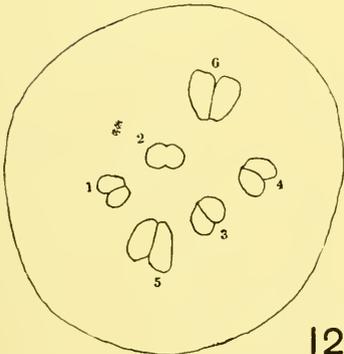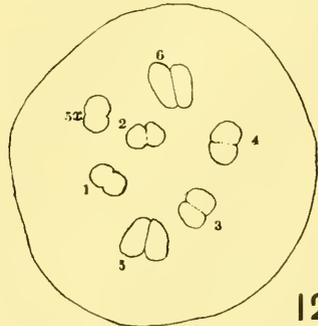
128 to 135 Tettigidea parvipennis Morse or parvipennis pennata Morse. First spermatocytes dividing. Tardiness and inequalities in division of the no. 6 chromosome in figures 132 and 134.
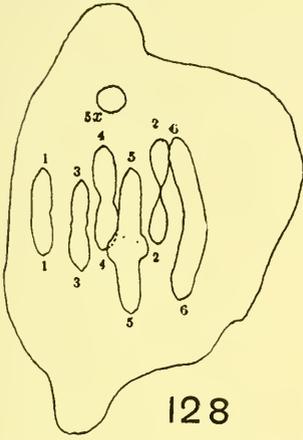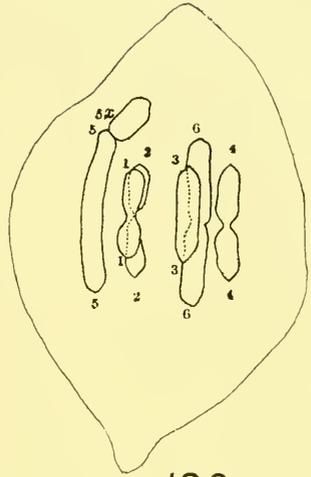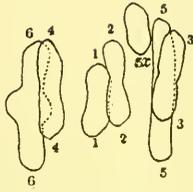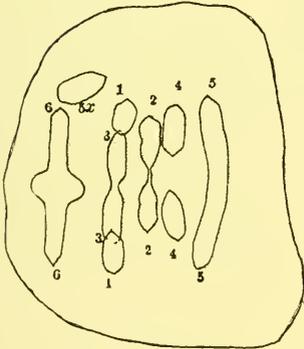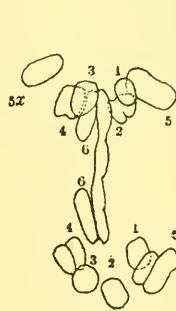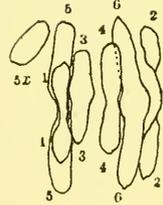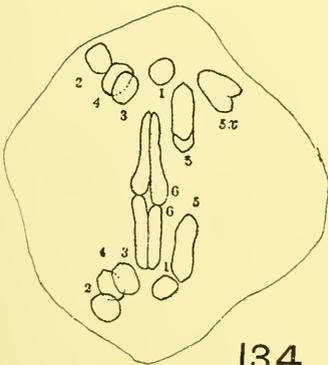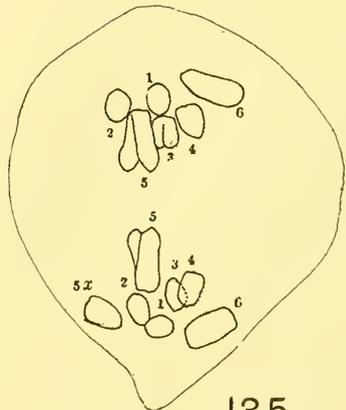
136 to 147   Acridium granulatus Scudd.

136 to 140   From walls of ovariole of ovary of one animal.   Fourteen chromosomes present.   Five long ones instead of four.   One long chromosome (1) is probably a mate to the normal no. 1.

141 to 147   First spermatocyte divisions from one male.   Note that all chromosome pairs are present and normal, except the smallest (no. 1), which is made up of a normal no. 1 and an abnormally long mate (1), from which it is separating.   The abnormal chromosome is constricted at a point as far from its distal end as the length of its no. 1 mate (figs. 142, 144, 146, 147).
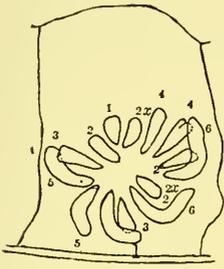
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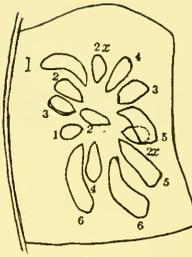
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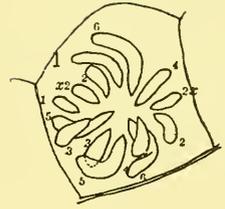
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139

140

141

142

143

144

145

146

147
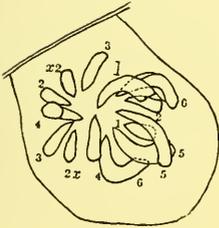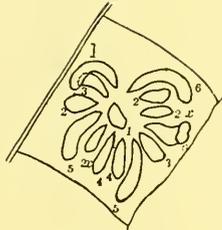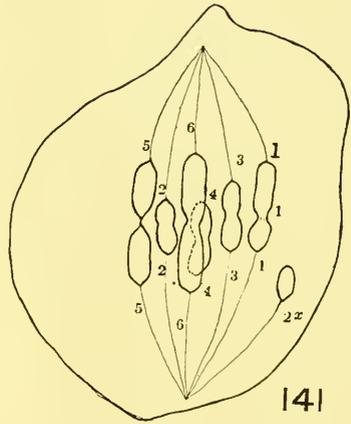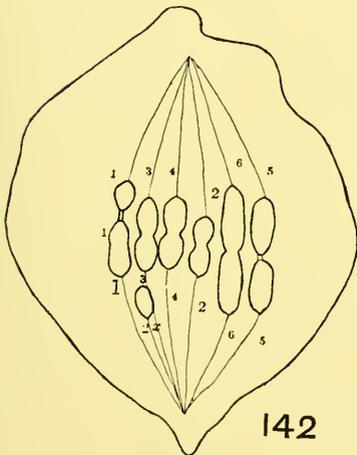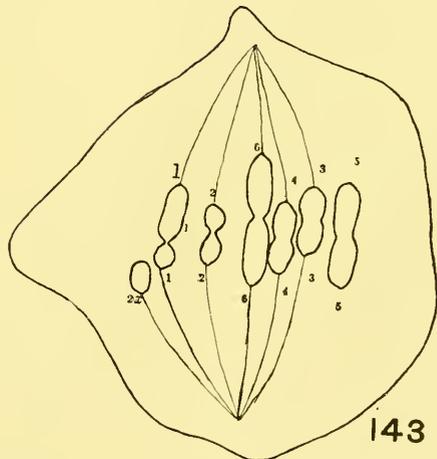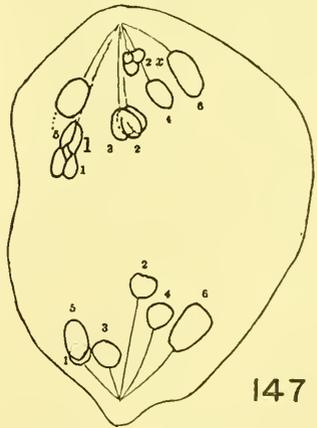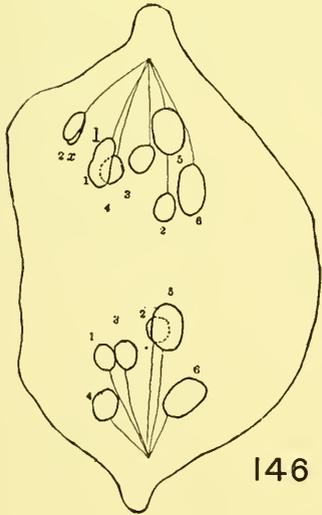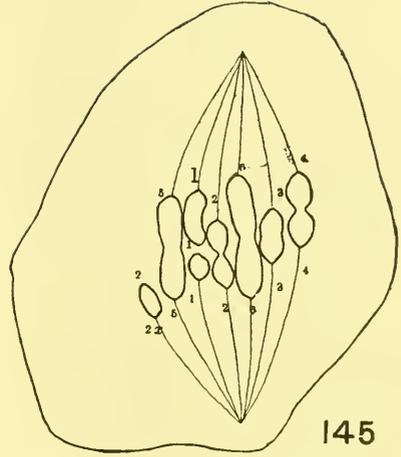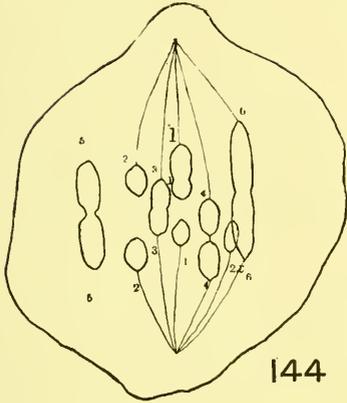
PLATE 18

148 to 160   Syrbula acuticornis Bruner.

148   Metaphase of spermatogonium.   Autosomes paired from 1 to 11 according to size.   Sex chromosome ($10x$) numbered 10 to show that it ranks tenth in size, being between the ninth and tenth autosomes; marked x to indicate its sex determining nature.
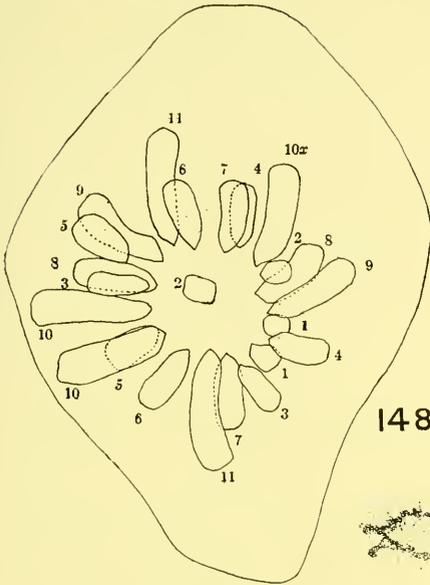
149 to 158   First spermatocyte stages.

149a   Early prophase of first spermatocyte stage h.   Four of the paired chromosomes coming out of parasynapsis.   x, x, indicate the points from which the attraction fibers will spring in the succeeding reduction division.

149b   Remainder of the nucleus of the spermatocyte shown in figure 149a (the following section).   In the cell shown in figures 149a and 149b the eleven paired autosomes and the sex chromosomes are all accounted for.

150a and 150b   Two successive sections of a spermatocyte at a stage (i), later than that shown in figure 149.   The four parts of the paired chromosomes are evident.   Chromosomes are condensing to enter the metaphase.

151   Portion of a nucleus showing the four parts of paired chromosomes 1, 2, 5, 11, and the continuation of the separation process following parasynapsis. Stage h.

152a to 152d   Stage i.   Individual chromosomes from different cells showing further continuation of the process of separation and the structure of the various paired autosomes about ready to enter the condensed metaphase stage. The attraction fiber knobs, i.e., the exconjugating knobs of the tetrad, are indicated by x, x.   152a would have formed a ring, 152b a cross, 152c a **V** with two knobs at the apex, and 152d a rod chromosome.
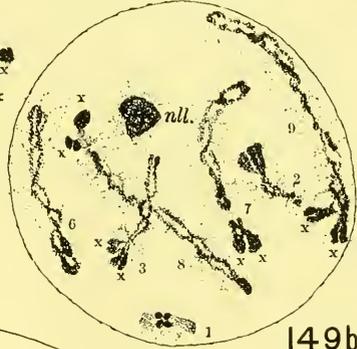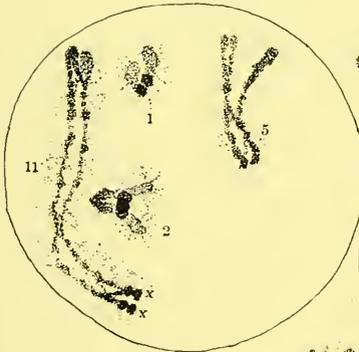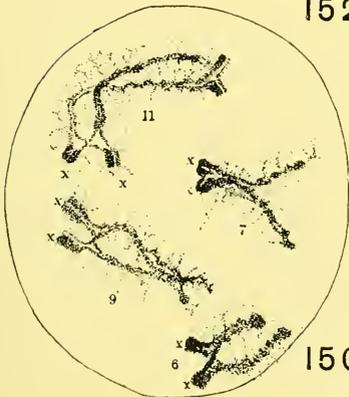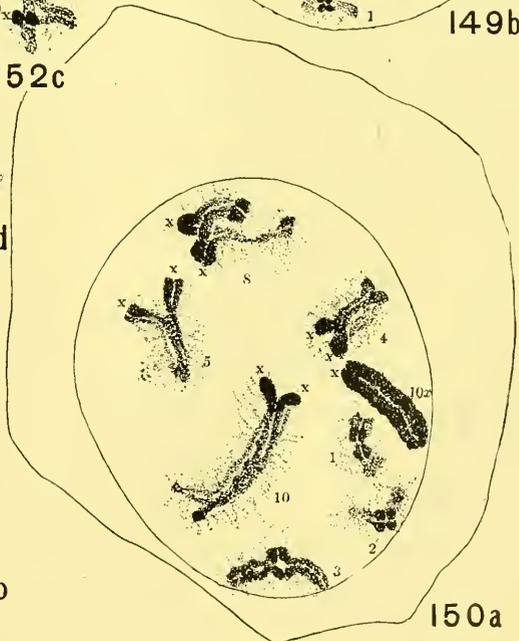
148

149a

152a

152b

149b

151
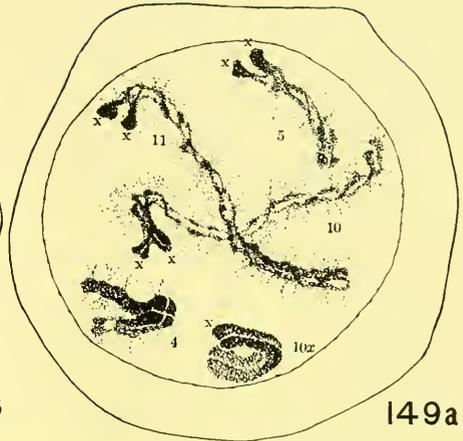
152c

152d

150b

150a

PLATE 19

(S. acuticornis)

153   A first spermatocyte in prophase. Stage *i*. Chromosome 11 about to form a ring with a pair of knobs at each end.   Near the knobs (x, x) the four parts of the chromosomes are easily distinguishable.

154   Five stages in the exconjugation of chromosome pair no. 4: a, the members are in parasynapsis; b, separation of the members has begun, and a second split, at right angles to the first, is now evident; c, separation almost complete; e, lateral view of same.

155a, 155b   Nucleus of later prophase, stage *j;* drawings of two successive sections.   All the chromosomes (complete) contained in the two sections.   Nos. 1 and 2, crosses; 3, a rod probably; 7 and 8, wide **V**'s with knobs at their apices; 4, 5, 6, 9, rings; 10, a four-knobbed ring; 11, a double ring.

156a to 156f   Stage *j*. Showing the primary (first maturation division) split (*I, I*), and the secondary (second maturation division) split (*II, II*) in various first spermatocyte chromosomes.   The proximal exconjugating portions of each pair are indicated by x, x.

157   Stage *k*.   First spermatocyte in metaphase; slightly oblique polar view; chromosomes in outline.   Nos. 1 to 3 rod-shaped tetrads similar to figures 156a and 156b, and possibly to no. 3 in figure 155b.   No. 4 is a cross, like no. 1, figure 155b, or no. 2, figure 155a.   Nos. 5, 9 are rings similar to 4 or 5, figure 155.   Nos. 6, 7, 8, 10 are knobbed **V**'s, like nos. 7, 8, figure 155, or figure 156c, or nos. 6, 8, figure 150.   No. 11 is a ring resembling figure 152a, or no. 11, figures 150, 151, or 153, or nos. 6, 9, figures 155, 156a.

153

154

155a

155b

156a   156b   156c

156d

156e

156f

157

158a, 158b S. acuticornis. Stage k. Lateral view of spindle and chromosomes in metaphase, similar to figure 157, from two successive sections. Nos. 1, 2 are rods; 3 approaches a ring in shape; 4, 5, 6 and 9 are crosses; 7 and 10, knobbed **V**'s, similar to no. 7, figure 155, or to figure 156; 8 is a view of the distal end of a knobbed ring similar to 10 in figure 155a; 11, side view of a ring similar to figure 156d.

159 S. acuticornis. Second spermatocyte in metaphase showing the relative lengths of the eleven autosomes. Sex chromosome absent from this cell. All chromosomes are split, though both parts do not always show.

160 S. acuticornis. Second spermatocyte in metaphase showing twelve chromosomes. The halves of 3, 7, 10, and $10x$ have begun to separate.

161 to 187 are from Chorthippus (Stenobothrus) curtipennis Bruner, one of the Truxalinae.
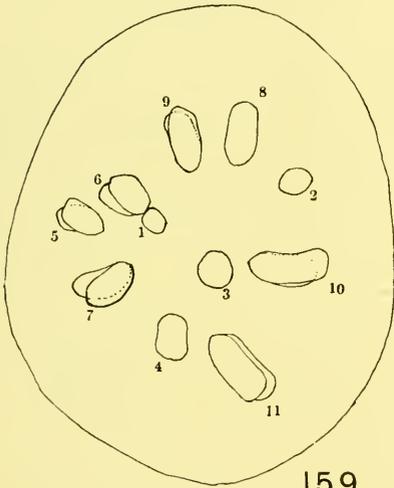
161, 162 Nuclei of spermatogonia in metaphase. The same size relations appear as in figure 148. The 5's are linked at their proximal ends with the 9's, the 7's with the 11's, and the 8's with the 10's.
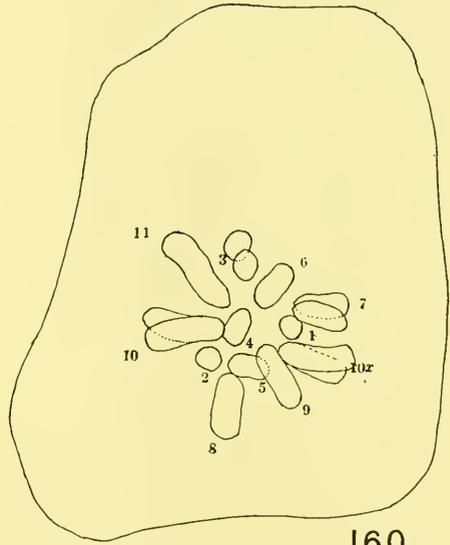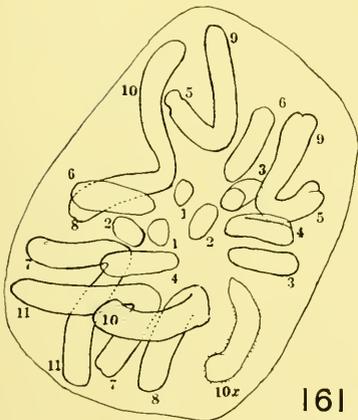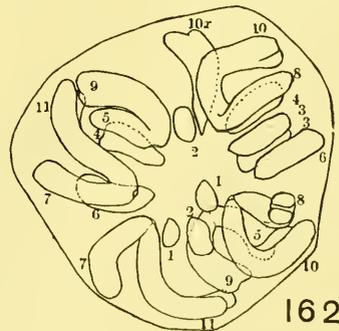
158a

158b

159

160

161

162
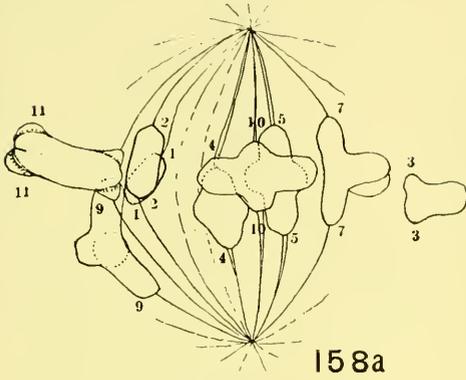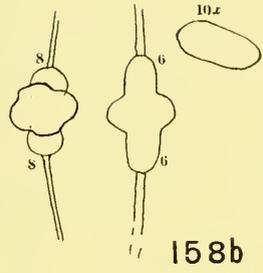
163 to 183 First spermatocyte.

163 Early prophase, stage *h*, parasynapsis still occurring. All chromosomes (nine) are present. To prevent confusion, nos. 2, 4, and 10*x* have been drawn outside the cell boundary, nos. 2 and 4 being moved out radially; no. 10*x* is from the nuclear-wall region at + near the figure 6. Distal and proximal ends are indicated by *dst.* and *prx.* respectively. x′ to x″″ are points at which spindle fibers become attached; they also indicate the proximal end or region. The numerals 5–9, 8–10 and 7–11 indicate the linked chromosomes 5 with 9, 8 with 10, etc. One-half (exconjugant portion) of the 7–11 in the proximal region (x′ to x‴) is included between the similar parts (x′ to x″) of the 8–10 chromosomes. The remaining parts of each chromosome are still in parasynapsis.

164 A slightly later stage. All chromosomes present. The autosome pairs still in parasynapsis. End views show each chromosome spireme to be split in two planes mutually perpendicular.

165 Stage *h*, similar to figure 163, also showing the nature of the 7–11 compound. At a and b, just outside the nuclear membrane, are drawn cross-sections of this chromosome at the two points opposite a and b. No. 4 lies near 10*x*, and, as in figures 149, 163, 164, 168, etc., is more condensed than the other autosomes.

166 Stage *h*, similar to figure 164, showing the four parts of a first-spermatocyte chromosome before exconjugation sets in.

167a and b similar examples. Stage *h*.

168 Prophase (stage *h*) approaching that of figure 150. The three multiples and all other chromosomes present. The proximal regions (x, x) are beginning the exconjugation process. The proximal part of one exconjugation portion of no. 4 is in contact with one end of the sex chromosome.

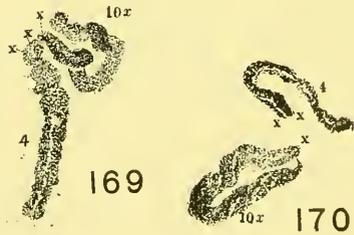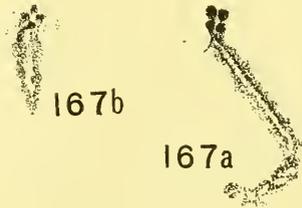169, 170 Show association of one end of the no. 4 pair (still in parasynapsis) with one end of the sex chromosome (10*x*).

163

164

165

166

167b

167a

168

169

170
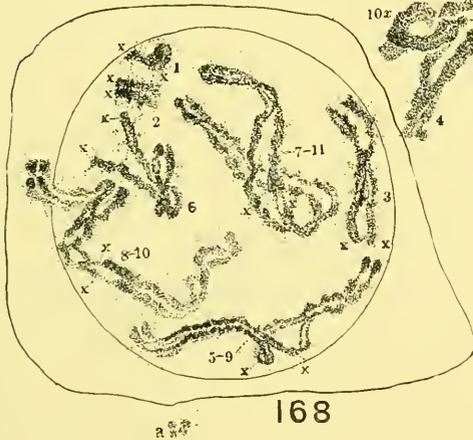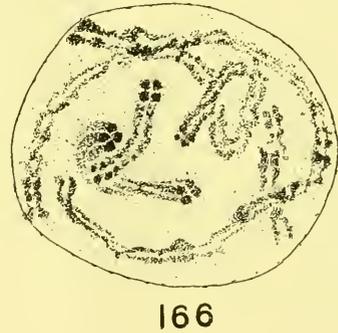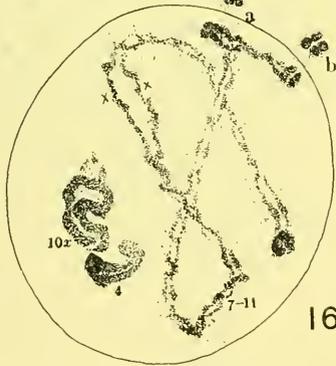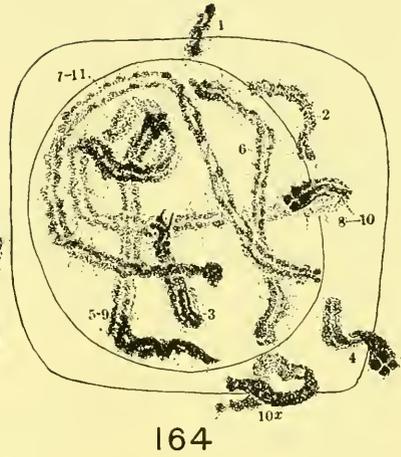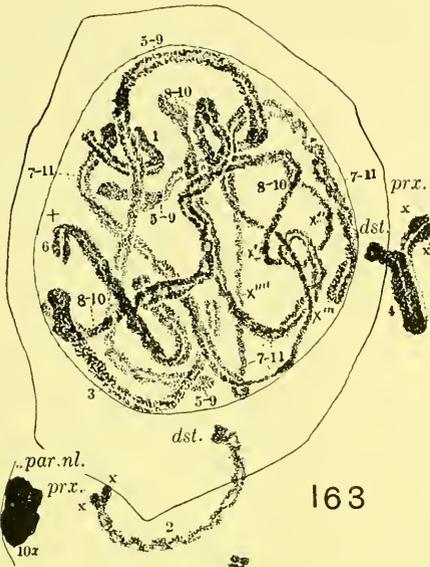
(C. curtipennis)

171   Shows conditions similar to those of figures 169 and 170.

172   Showing the same association, also the relative condensation of the no. 4 and 10x chromosomes and that of the other autosomes (no. 6) in the same cell.

173a, 173b   A prophase similar to figure 150, showing a complete set of chromosomes.   7–11 is displaced and drawn as 173a to make room for the other chromosomes.   Note the position and the stage of condensation of nos. 4 and 10x.

174   Stage i.   Prophase similar to figure 155.   The junction of 7 with 11, 5 with 9, and 8 with 10 is shown by constriction at x, x in each case.

175   Stage i.   The 7–11 multiple-chromosome.

176   Stage i.   Junction of 8 with 10 shown at x, x by constrictions.   x, x correspond to the apices of the 8–10 V's of figures 161, 162.

177   A 7–11 pair enclosing a 5-9 pair.   Stage i.
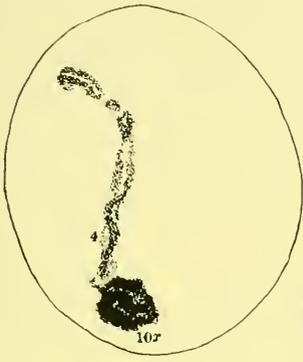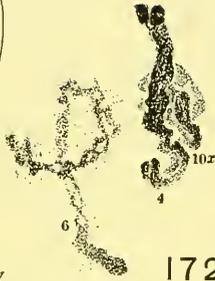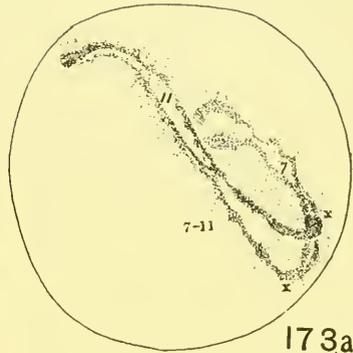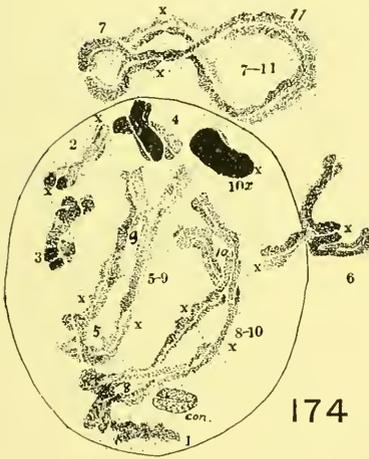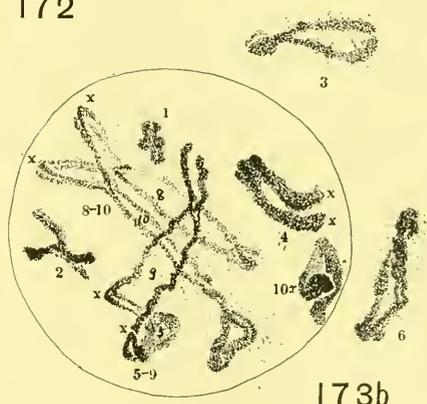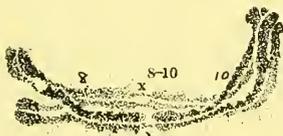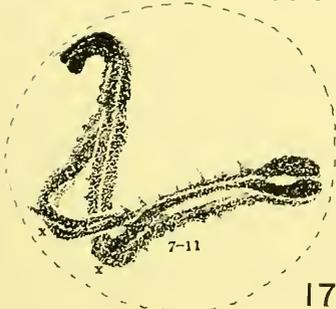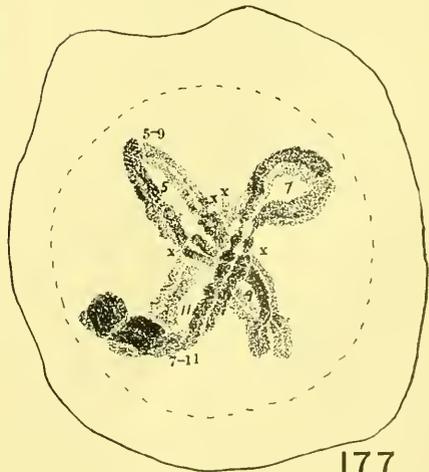
(C. curtipennis)

178a, 178b Stage *j*. Complete set of chromosomes from a late prophase. Compare each chromosome with those of figure 155. The four parts of each are apparent.

179 Stage *k*. Lateral view of metaphase similar to figures 157 and 158. The tetrad no. 2 is lacking from the section. No. 1 is a rod; no. 4, a **V**; no. 5, a **V**; no. 6, a two-knotted ring; nos. 7 and 8, similar to no. 8 of figure 157; no. 9, a ring; no. 10, similar to no. 10 of figures 157 and 158; no. 11, a four-knobbed ring similar to no. 11 of figures 157 and 158.

180a, 180b Stage *k*. A similar stage, all chromosomes present obtained from two sections. Note constrictions at x, x in 180b.

181 Stage *l*. E-shaped figures resulting in the separation of the members of the 7–11 and 8–10 pairs in the first maturation division. The no. 7 components, shorter than the no. 11 components, separate before the longer companions (no. 11) do. The same is true for chromosome 5–9.

182a, 182b, Stage *k*, 182c Stage *l*. Stages in the separation of the chromosomes 7–11 from their mates in the 7–11 multiple during the first spermatocyte division.

183a (stage *k*) and 183b Similar stages in the 8–10 multiple.

WM. REES B. ROBERTSON
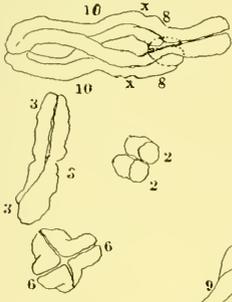
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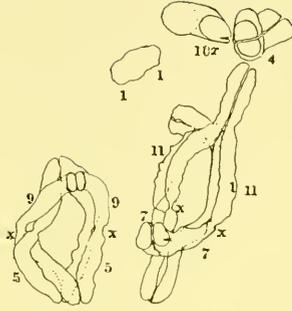
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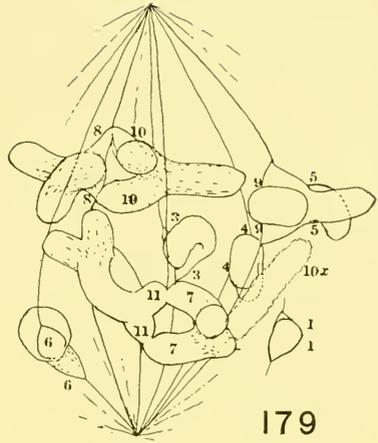
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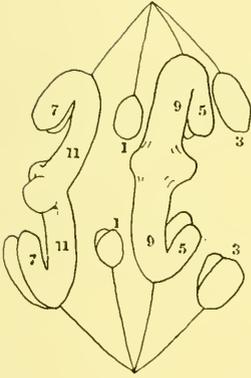
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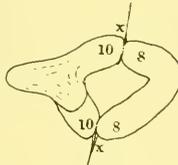
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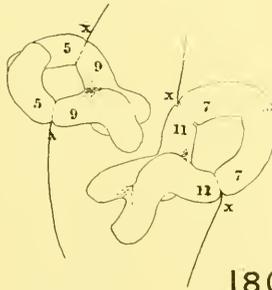
180b

180a

182a

182b

182c

183b

184

185

186

187

188

189

PLATE 25

190 Spermatogonium of Chortaphaga viridifasciata, one of the Oedipodinae, after Davis ('08). Same number of pairs of autosomes. Sex chromosome ranked $8x$.

191 Spermatogonium of Dissosteira carolina, one of the Oedipodinae, after Davis ('08). Eleven pairs of autosomes plus one sex chromosome $(9x)$.

192 Spermatogonium of Melanoplus femoratus, one of the Acridiinae, after Davis ('08). Eleven pairs of atuosomes plus sex chromosome $(10x)$.

In figures 148, 159 to 162, 184 to 192, note that all subfamilies have three extremely small chromosomes or pairs, and three extremely large ones with a somewhat similarly graded series between.

193 to 202 from Locustidae.

193 Spermatogonium of Steiroxys trilineata after Davis ('08). Thirty-one rod-shaped chromosomes.

194 to 201 After Woolsey ('15).

194 Spermatogonial chromosome group from Jamaicana subguttata Walker. Thirty-five rod-shaped chromosomes, paired. Sex chromosome is $18x$.

195 First maturation division of same species, showing seventeen first spermatocyte pairs of autosomes in the process of reduction. Sex chromosome $(18x)$ going over undivided to one pole.
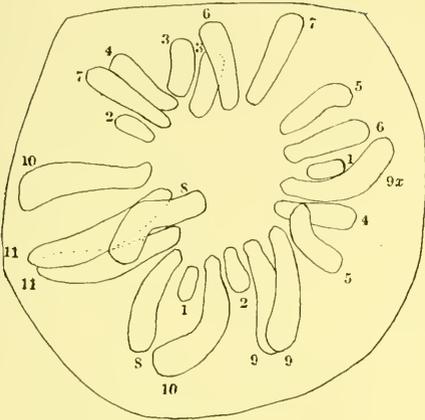
191

190

192

193

194

18x

195
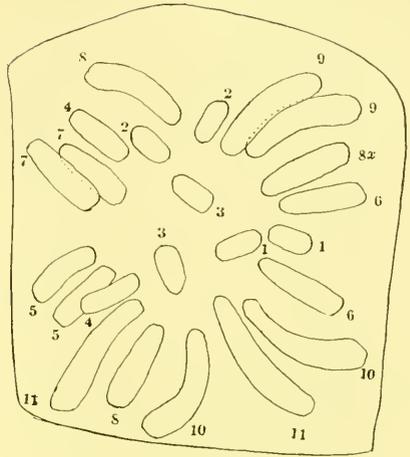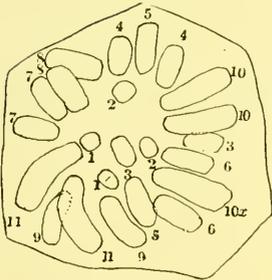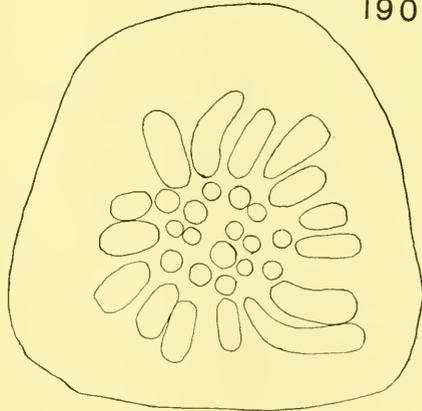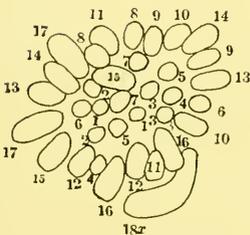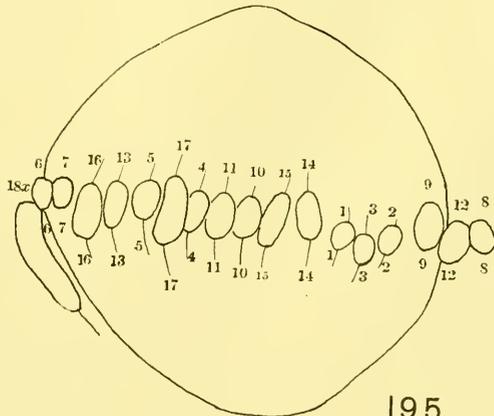
PLATE 26

196 Spermatogonium of Jamaicana subguttata Walker, showing one member of the no. 14 pair linked with one member of the no. 16 pair. Thirty-two rod-shaped chromosomes, plus one V-type, plus the sex chromosome, are present.

197 Prophase of first spermatocyte of same animal showing fifteen tetrads plus the V bi-tetrad plus the sex chromosome.

198, 199 Lateral views from same animal showing the separation of the short no. 14 and long no. 16 limbs of the V from their no. 14 and no. 16 rod mates. Points of separation marked by the constrictions.

200 Spermatogonium from Jamaicana unicolor Bruner, showing two V's of equal size. Thirty rod-shaped chromosomes, plus two V's, plus the sex chromosome, are present.

201 Prophase of first spermatocyte from same animal showing the sex chromosome ($18x$), the fifteen rod-tetrads, and the large ring-shaped bi-tetrad resulting from the separation of the V's in exconjugation. Note the unequal length of the 14, 14 and the 16, 16 sides of the ring.

202 Spermatogonium of Steiroxys trilineata, after Davis ('08), showing the presence of twenty-six rod-shaped chromosomes plus one sex chromosome. Compare with figure 193 in number.

203, 204 Of Gryllis domesticus, after Baumgartner ('04).

203 Spermatogonium, showing twenty autosomes plus one V-shaped sex chromosome (x). Eight of the autosomes are V-shaped with apices turned toward the center of cell plate.

204 Second spermatocyte, ten autosomes plus the sex chromosome. At least four of the autosomes are V's with apices turned toward the center of the plate.
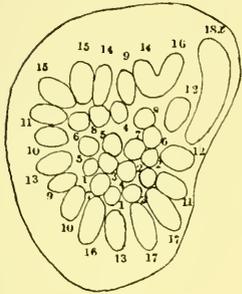
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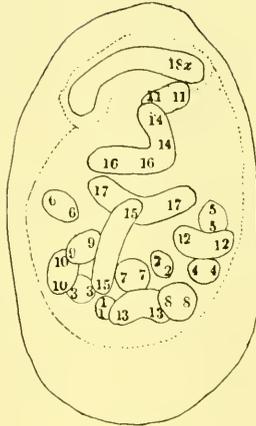
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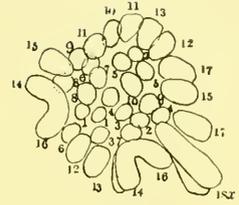
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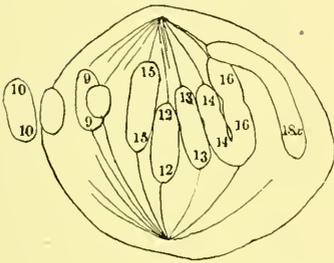
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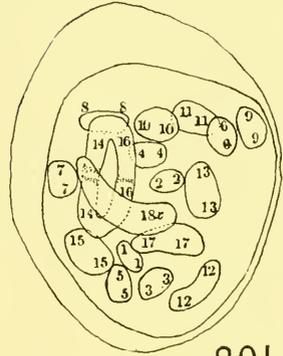
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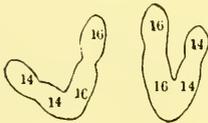
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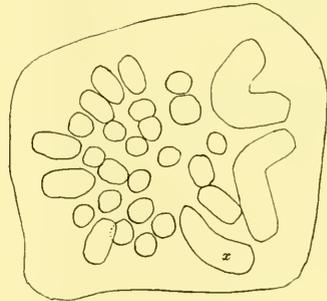
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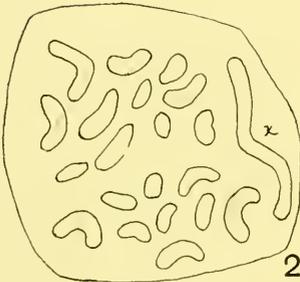
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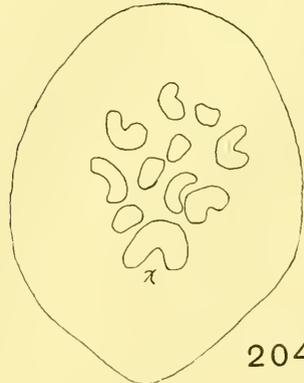
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THE STRUCTURE AND DEVELOPMENT OF A MYXO-SPORIDIAN PARASITE OF THE SQUETEAGUE, CYNOSCION REGALIS[1]

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CONTENTS

I. INTRODUCTION

During the course of a somewhat extended study of the Myxosporidian parasites of the marine fishes of Beaufort, N. C., an undescribed species was found in the urinary bladder and ureters of the common squeteague, Cynoscion regalis, which showed so many points of exceptional interest that an extended investigation was made of its structure and development.

[1] Published with the permission of the Commissioner of Fisheries. Contribution from United States Fisheries Biological Station, Beaufort, N. C.

II. MATERIAL AND METHODS

The Myxosporidian, which I have named Sphaerospora dimorpha, is abundant in the urinary bladder of Cynoscion regalis, extending into the Wolffian ducts in large numbers, but I have never found it in the kidney tubules proper. The parasite occurs as two very different forms, one disporous, the other polysporous, although both forms are usually present together in the same host. A large number of fish were examined and in no case was the bladder found to be entirely free from infection although there was great variation in the abundance of the parasites. The results incorporated in this paper are based on the study of a large amount of material, both fresh and preserved.

For studying the living trophozoites a small amount of material was taken from the bladder in a pipette and transferred to a slide where it was studied at once.[2] To prevent evaporation the cover was at first ringed with vaseline, but later paraffine of a low melting point was found to be much superior for this purpose and was thereafter used exclusively. Under these conditions it was possible to keep the trophozoites for several hours without any appreciably bad results. However, after six or eight hours the trophozoites usually showed distinct signs of degeneration, probably due to the rapid increase of bacteria in the preparation. Attempts were made to grow the trophozoites on agar culture media but without success, although they lived somewhat longer under these conditions than on the slide.

In studying preserved material both dried smears and sections were used. In preparing the smears a small amount of material was removed from the bladder with a pipette and smeared thinly over a coverglass which was then exposed to osmic vapor for 30 to 60 seconds, after which the preparation

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[2] Since the parasites are commonly attached to the lining of the urinary bladder it was found in practice that to insure getting a large number of trophozoites it was first necessary to allow most of the urine to escape. This was done by slitting the wall of the bladder with the scissors, allowing the urine to flow out, and then by applying the mouth of the pipette to the inner wall and forcibly sucking up the remainder of the contents the parasites could invariably be obtained in large numbers.

was dried, then placed in absolute alcohol 15 to 30 minutes, then washed in distilled water to remove any soluble substances present in the urine which might interfere with the stain. After washing the preparations were stained with Giemsa's stain over night, decolorized with acetone and then mounted in neutral balsam or damar. The latter was found to be preferable since the stain was much more permanent than in balsam.

For sectioning, the infected tissues were removed from fish which had not been dead more than 5 to 10 minutes, placed in Worcester's formol corrosive acetic fluid for several hours. After washing and dehydrating in the usual manner the tissues were imbedded in paraffine and sectioned. The sections were stained with iron-hematoxylin and Congo red. This method gave excellent results, both the nuclear and cytoplasmic structures being well preserved. Material killed in Schaudinn's fluid and also in Hermann's fluid was inferior to that obtained with Worcester's fluid.

Most of the work was done at the Bureau of Fisheries Laboratory, Beaufort, N. C. during June, July and August, 1911 and 1912.[3]

III. DESCRIPTION OF TROPHOZOITES AND SPORULATION

1. Disporous form

a. Living trophozoites. When the contents of the urinary bladder are placed on the slide the trophozoites can usually be found in large numbers. In most cases during June, July and August both the younger stages previous to spore formation and older trophozoites containing spores in all stages of development, are abundant.

The non-sporulating trophozoites are very distinctly amoeboid but sluggish in their movements (fig. 1). When attached to the lining of the bladder they are usually somewhat rounded and closely applied to the free, rounded ends of the epithelial cells, with one to several pseudopodia extending down between

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[3] Grateful acknowledgment is hereby made to the Bureau of Fisheries for the opportunity to do this work.

the cells (fig. 21). On being transferred to a slide they become actively motile, often sending out pseudopodia in several directions at once, and soon became very irregular in shape.

The ectoplasm is easily distinguished as a homogeneous, hyaline layer surrounding the endoplasm which is filled with rather indistinct vacuoles (fig. 1), separated from each other by a denser, homogeneous substance which has much the same appearance as the surrounding ectoplasm. In fact the endoplasm gradually merges into the ectoplasm by the vacuoles gradually becoming less abundant until in the latter they entirely disappear. Usually the endoplasm also contains a few yellowish, refractive globules, evidently composed of fat since they stain intensely with Sudan III. These globules vary considerably in size and numbers, being, in general, more abundant in older individuals; although there is considerable variation in this respect in trophozoites of approximately the same age. In some cases, even in full grown individuals, there are only a few fat globules present while in others they may be very abundant.

Occasionally the endoplasm contains one or more erythrocytes which are evidently obtained from the blood of the host and are ingested by the parasites (fig. 2). I am convinced that the erythrocytes are actually taken in and digested by the trophozoites since I have often found individuals, both in fresh and preserved material, in which they were unquestionably inclosed in the endoplasm. Erythrocytes in various stages of disintegration occasionally occur within the endoplasm so there can be little doubt that this species actually utilizes solid food. Cohn ('96) found that erythrocytes were ingested by Myxidium lieberkuhni Bütschli, a parasite of the urinary bladder of the pike, but his statements have been questioned by later writers. The evidence in the case of S. dimorpha is, however, so clear that there can be little doubt that this species forms a notable exception, as regards the character of its food, to most of the Myxosporidia. On the other hand, individuals containing erythrocytes are too few to justify the conclusion that this is the only means of obtaining food. In all probability they absorb

liquid food as well. There is also evidence that the tropho-
zoites may ingest any small, solid particles present in the urine.

I have been unable to find any case where the bladder epi-
thelium showed appreciable injury by the parasites, although
the presence of blood corpuscles in the urine would indicate
that this must occasionally occur.

The size of the full-grown, vegetative trophozoites varies
somewhat, the average diameter being from 25 to 30μ. As spor-
ulating trophozoites were found to have approximately the same
diameter this is believed to be the maximum size reached by this
form.

Large numbers of trophozoites were observed in which the
spores were in various stages of development. These showed
practically the same characteristics as the vegetative forms,
except that most of the endoplasm is used up in forming the
spores, the fat globules which remain being crowded to one side
between the two spores (fig. 44). Trophozoites in which the
spores were matured usually disintegrated very quickly after be-
ing placed on the slide, thus setting the spores free.

The spores (figs. 45 and 46) are approximately spherical with
an average diameter of about 15μ. In a few cases the diameter
was found to be slightly greater in one direction than in others,
but this is not believed to be a constant character. The spores
contain two polar capsules, each having a diameter of 4.5μ.
The length of the polar filaments after extrusion varies from 27
to 35μ. The sporoplasm is plainly seen within the spore as a
rounded, finely granular mass below the polar capsules. Num-
bers of fat globules are often present in the spore, part of them
being inclosed within the sporoplasm. The sutural line, formed
by the junction of the halves of the spore membrane, is not
straight, as is the case in many species of Myxosporidia, but
takes a characteristic sinuous course around the spore which
can best be understood by consulting figure 46. Usually one
valve of the spore membrane is slightly smaller than the other
into which it appears to telescope.

b. Preserved material. In giving an account of the results
obtained from the study of stained material it will simplify mat-

ters to begin with the small, mono-nuclear trophozoites. This stage occurs only rarely in my material, and is characterized by the relatively large amount of dense, finely granular cytoplasm containing a large, well defined nucleus (figs. 8 and 9). Within the nucleus the chromatin forms a well defined network composed of irregular granules varying greatly in size and especially abundant around the periphery. A conspicuous nucleolus is always present and often (especially in strongly decolorized specimens) can be seen to be composed of a deeply staining outer portion enclosing a central mass which stains less intensely. Usually—possibly in all cases—the nucleolus is attached to the nuclear membrane, and sometimes, as in figure 9, the side in contact with the membrane is somewhat separated from the rest with which it is connected by a faintly staining substance.

The next stage in the development of the trophozoites is shown in figures 10 to 15. The trophozoite now contains two nuclei of equal size and similar appearance, each surrounded by a layer of dense cytoplasm which, in most cases, can be more or less clearly differentiated from the less deeply staining material composing the remainder of the individual. The denser, more deeply staining area of cytoplasm around each nucleus is, however, apparently not surrounded by a definite membrane at this time. While it can usually be more or less easily distinguished from the surrounding cytoplasm, in some cases the two merge together so gradually that no distinction can be made (figs. 12 and 16). That even in such cases there is a specially differentiated area around each nucleus is clearly shown in figure 26, where, owing to a defect in fixation, the cytoplasm immediately surrounding the nucleus has shrunken away from the adjacent endoplasm. These two nuclei are, I believe, derived by mitotic division from the single nucleus of the preceding stage. In a dried smear stained by Giemsa's method, I found a mononuclear trophozoite in which the nucleus was dividing mitotically. Moreover, the bi-nucleate stage is often scarcely, if any larger, than trophozoites which contain but a single nucleus and thus can scarcely be formed by the fusion of two such trophozoites. Although the two nuclei are similar in size and appearance, yet

each has a very different later history. One becomes the vegetative nucleus and, in most cases, undergoes no further division, while the other divides a number of times to form the spores.

At a little later stage each trophozoite contains three nuclei, one being considerably larger than the other two (figs. 16 to 21). The two smaller nuclei are derived from one of the nuclei of the preceding stage by mitotic division while the other nucleus remains undivided. The latter gradually increases in size, the specially differentiated area of surrounding cytoplasm becoming indistinguishable from the rest of the trophozoite. This forms the vegetative nucleus and ordinarily undergoes no further changes, but rarely may, in later stages, divide amitotically (fig. 30). This division of the vegetative nucleus is very exceptional and only in rare cases have two vegetative nuclei been found in the same trophozoite. The two smaller nuclei are the generative nuclei and soon divide by mitosis to form four nuclei of equal size (figs. 23 and 24). One of the generative nuclei usually divides before the other (figs. 20 to 22) so that trophozoites with but four nuclei are common. As a result of these divisions trophozoites are formed with five nuclei, one larger vegetative nucleus, and four generative nuclei of equal size, each surrounded by a denser more deeply staining area of cytoplasm. The generative nuclei then become grouped in pairs (fig. 24), each pair forming a sporoblast. At the same time one of the nuclei of each pair divides mitotically, resulting in sporoblasts with three nuclei, one nucleus in each being considerably larger than the other two (figs. 25, 27, 28, 29 and 32). Trophozoites with two sporoblasts, each with three nuclei, are more abundant in my preparations than any other stage. At this time the cytoplasm immediately surrounding the sporoblast nuclei is much denser and more clearly defined than at any preceding stage, and is sharply marked off from the surrounding cytoplasm which now stains less deeply than before. Each sporoblast is, I believe, composed of three distinct cells corresponding to the three nuclei, but in many cases the cells are so closely associated that they can only be distinguished with difficulty, and sometimes not at all. In all cases during this and later stages the

two sporoblasts are entirely distinct, and are often quite widely separated.

The later history of each sporoblast is the same, although one usually lags a little behind the other in development. The two smaller cells divide by mitosis, one usually a little after the other (figs. 29, 33 and 34) forming sporoblasts with five cells, usually lying in approximately the same plane and arranged in a definite manner (figs. 30, 31, 35 and 36). The division in this case is unequal, one of the daughter cells from each mother cell being slightly larger than the other. These two cells later form the capsulogenous cells which give rise to the polar capsules; the two adjoining smaller cells fuse to form the sporoplasm, while the large cell at the end opposite the capsulogenous cells, divides by amitosis into cells which later form the valves of the sporocyst. In this division the nucleolus divides first, after which the nucleus divides by a simple constriction (figs. 37 and 38). Thus in each three-celled sporoblast the larger of the three cells is destined to produce the parietal cells which form the valves of the sporocyst, while each of the smaller cells will give rise to one of the capsulogenous cells and one of the sporoplasm cells.

Up to this time the resting nuclei show a similar structure throughout the different stages, but shortly after the division of the sporoblast into six cells the nuclei present a quite different appearance (figs. 39 and 40). Definite nucleoli are no longer to be distinguished, but are probably represented by several deeply staining masses closely apposed to the nuclear membrane. These masses are probably composed chiefly of chromatin, the achromatic part of the nucleolus having disintegrated. Throughout this and later stages there is a marked tendency in all the nuclei for the chromatin to collect in larger and larger masses.

At this time there is also a general rearrangement of the cells in each sporoblast, each cell assuming the position relative to the others which it occupies in the mature spore. During the formation of the spore all the nuclei decrease in size and stain more deeply as a result of the concentration of the chromatin in larger masses. The two parietal cells become flattened and

enclose the others, while the sporoplasm cells fuse into a common mass of protoplasm; the nuclei, however, remain distinct even in the mature spore (figs. 41 to 43). The capsulogenous cells can easily be distinguished by the fact that the cytoplasm becomes condensed at one side of the nucleus and contains numerous minute granules which stain deeply with the plasma stain (fig. 41). A little later a small, rounded, deeply staining body appears in the concentrated cytoplasmic mass (fig. 42). At a somewhat later stage this body becomes differentiated into a clear space surrounding a central, deeply staining part which is evidently the developing thread of the capsule (fig. 43).

Sporoblast cells undergoing mitotic division are common in sectioned material so that the details of the process can be easily worked out. During the prophase (figs. 48 and 49) the chromatin forms a coiled spireme. Apparently there is more than one of these chromatin threads, although there are certainly not as many as there are distinct chromosomes later. Usually the spireme can be traced into one or more irregular masses of chromatin imbedded in achromatic material. These masses are probably disintegrating nucleoli, the chromatin of which is taking part in the formation of the chromosomes. As indicated on page 338—the nuclei are composed of two distinct materials— an outer portion which shows a great affinity for chromatin stains and is, in all probability, chromatin; and an inner portion which stains less readily and is evidently composed of plastin.

During the metaphase the chromosomes become arranged around the equator of the spindle in the usual manner (figs. 50 to 52). The chromosomes are usually so closely crowded together as to make it difficult to count them with certainty but polar views of a few exceptionally favorable cells show that there are, without doubt, six chromosomes. As regards size these chromosomes can be readily grouped into three pairs, one of which is considerably larger than the other two, one smaller, while the third pair is intermediate in size. The spindle is poorly developed and at no time have I been able to find any trace of centrosomes. Figure 53 shows an early, figure 54 a later anaphase. In the latter the well-defined remains of the nucleolus

can be distinguished at one side of the spindle.   In many cases
as in figure 53 the nucleolus has entirely disappeared at this
time and I have never been able to distinguish it at a later stage.
In all cases, when present, the nucleolus lies at one side, and at
some distance from the spindle, never on it.   Figure 55 shows a
telophase in which the interzonal filaments can be plainly dis-
tinguished and there is a slightly developed 'zwischenkörper.'
In some cases the interzonal filaments may persist for a short
time after the daughter cells have otherwise entirely separated.

2. Polysporous form

a. *Living trophozoites*.   In addition to the forms described
above there are almost invariably present a number of tropho-
zoites which have a very different appearance.   These tropho-
zoites, which are usually much larger than the disporous form,
are sometimes relatively rare, in other cases they were found to
be equally, if not more abundant than the smaller form.   I
am uncertain whether or not they are ever entirely absent.   Ac-
cording to my notes a few fish examined during the early part
of the investigation contained none of this type, but I do not
feel at all certain that they were not present in small numbers,
since in that case they could easily be overlooked.   At first I
considered that the larger trophozoites were specifically distinct,
but was later forced to the conclusion that both the large and
small types of trophozoites are but different forms of the same
species.   The reasons for this conclusion will be given later.
The polysporous trophozoites have a very characteristic ap-
pearance, and in all but the youngest stages can readily be dis-
tinguished from the disporous form already described.   When
attached to the inner lining of the bladder they are usually elon-
gated and cylindrical, but considerably larger at the base where
they are attached to the epithelium (figs. 61 and 62).   The free
end of the body is commonly drawn out a long, tail-like process,
rounded at the end (fig. 56).   The size varies greatly.   Some
are no larger than the disporous trophozoites while one of the
largest measured was 575μ long with a maximum diameter of

90μ.   Another individual measured 270μ long with a maximum diameter of 60μ.   However, since they vary so greatly in shape and, especially, in the length of the slender free portion of the body, measurements can only give a very inaccurate idea of the size (text figs. A and B).
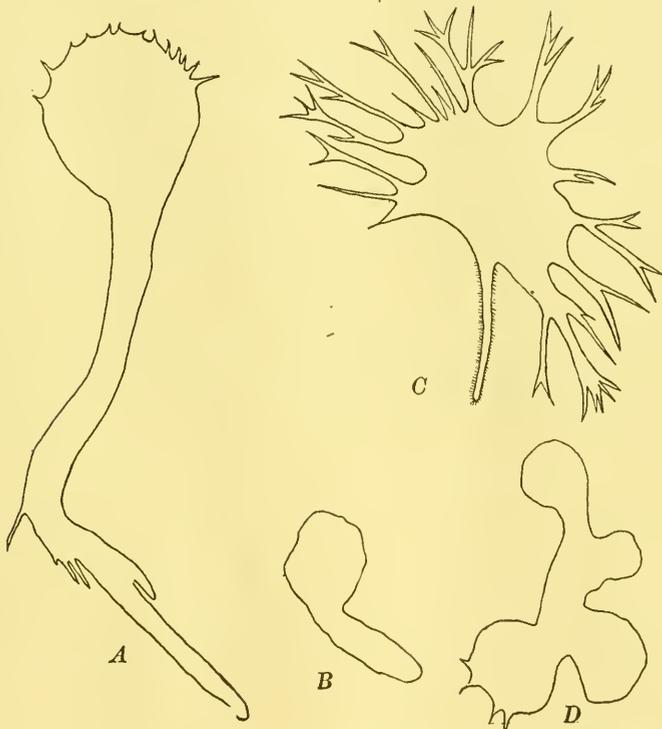
Text-figures A to D   Various forms assumed by polysporous trophyozoites after being removed from the urinary bladder. $\times$ 350.

The body is covered with short hair- or bristle-like processes of the ectoplasm which are larger and more abundant near the free end (figs. 56 and 60 to 62).   These processes are similar to those which cover the body of Myxidium lieberkühni and the writer has seen similar processes in several other species.   They are often well developed in Chloromyxum leydigi, common in the gall bladder of many species of sharks and rays.   While not

movable these processes are readily absorbed, and may later
be reformed. When the trophozoites are attached to the blad-
der epithelium the ectoplasmic processes usually cover the entire
free surface of the body, but after being placed on the slide the
processes are usually rapidly absorbed, until after a short time
they may disappear over the entire body.

The trophozoites are attached to the epithelium of the urinary
vessels by a specially modified part of the larger end of the body.
The ectoplasm, which is here more abundant and probably denser
than on other parts of the body, forms irregular, thin, plate-
like processes (pseudopodia) which extend in between the outer
ends of the epithelial cells (figs. 61 to 64). Between the plate-
like psuedopodia are depressions which receive the free, rounded
ends of the epithelial cells. Although the trophozoites thus be-
come very closely applied to the epithelium, I have been unable
to find any evidence that the cells are injured thereby. This is
the more remarkable when we consider that in cases where the
parasites are very abundant almost the entire epithelium may be
covered with them.

Often the trophozoites are more or less completely covered
with small granules and débris of various kinds. In some cases
almost the entire surface of the body is thickly covered with
extraneous particles, and even erythrocytes can occasionally
be seen clinging to it. This viscid character of the ectoplasm is
undoubtedly due to the short, bristle-like processes, since parti-
cles only adhere to the body where these are present.

The ectoplasm is abundant, clear, homogeneous or faintly
granular and usually covers the entire body but is more abundant
around the enlarged basal· portion (figs. 56 and 60). The en-
doplasm is distinctly granular and, usually, more or less vacuo-
lated. Small fat globules are commonly present but may be
entirely absent in the smaller individuals, although abundant
in the larger trophozoites. In addition to the fat globules,
small, irregular, refractive granules are usually rather abundant
in the endoplasm. Occasionally a few yellowish crystals can be
seen, but these are never abundant and in the majority of in-
dividuals are entirely wanting. They are apparently similar

to those described as hematoidin in Myxidium lieberkühni, but they always lie free in the endoplasm and are never enclosed in fat globules as in that species.   In many cases erythrocytes were observed in the endoplasm (fig. 57).   In fact they are more frequently found in the polysporous than in the disporous trophozoites.   As in the disporous forms a careful study of both living trophozoites and sections has convinced me that erythrocytes are actually enclosed within the endoplasm and are not always simply adhering to the exterior of the body as often happens. This is clearly shown in the case of sections where there can be no doubt as to their position.   Many of the refractive and other granules in the endoplasm are indistinguishable from those found floating freely in the urine and attached to the exterior of the body, and have probably been ingested in the same way as the erythrocytes.

Very often the endoplasm contains several rounded bodies (fig. 59) composed of an outer hyaline layer surrounding a granular central portion which contains numerous refractive granules. As will be described later, these bodies are gemmules which make their way out of the parent and develop into daughter tropozoites.

Occasionally a large trophozoite was found containing a number of spores which were in all respects identical with those formed by the small disporous individuals.   In the large trophozoites, however, there are always more than two spores and sometimes they are so abundant as almost to fill the entire body. Large trophozoites containing spores were never abundant and many fish were examined in which no sporulating individuals of this type were found.

Soon after being removed to the slide the trophozoites begin to send out numerous long, slender, branched pseudopodia from the large anterior end of the body.   These pseudopodia when first formed are composed entirely of ectoplasm but later endoplasm may flow into the proximal portion.   Still later, especially in smaller individuals, nearly the entire body may take part in the formation of pseudopodia so that the trophozoites may assume very irregular and grotesque forms (text figs. C and

D).   In several cases after being on the slide for some time they
became so irregular in shape that the body was divided into
two or more parts connected only by a small strand of proto-
plasm, and in two or three instances the parts were observed
to separate by moving in opposite directions until all connection
was destroyed (text figs. E to G).   This is evidently a case of
plasmotomy but whether it occurs under normal conditions
I am unable to say.

    After being on the slide for several hours the branched, atten-
uated pseudopodia disappear and are replaced by a few rounded,

    Text-figures E to G   Division of polysporous trophozoites after being on the
slide for some time.   × 350.

lobe-like pseudopodia (text fig. D), the trophozoites by this
time having become practically motionless.

    In a few cases a phenomenon was observed which is appar-
ently similar to that described by Cohn ('96) in Myxidium lieber-
kühni, which he believed to be a method of reproduction.   Several
of the largest trophozoites, after being placed on the slide for
some time, formed numbers of rounded, bud-like protuberances,
varying greatly in size, along the posterior part of the body
where the ectoplasmic hair-like covering was well developed
(fig. 58).   These rounded processes were, in most cases at least,
composed entirely of ectoplasm.   In a very few of the larger
processes a faintly granular central portion could be distinguished
which may possibly have been endoplasm.   Many of the proc-
esses were observed to change their position on the trophozoite,

indicating that they were simply adhering to the body by means of the viscid ectoplasmic covering. The formation of these bud-like structures was only rarely observed and was, I believe, an abnormal process due to the removal of the trophozoites to the slide. They were never observed until after the trophozoites had been on the slide for some time, usually several hours. No trace of bud formation has ever been observed in the preserved material, where great care was always taken to prevent degenerative changes. Moreover the fact that these bodies were so variable in size and, with few exceptions, were almost certainly composed entirely of ectoplasm, would indicate that they can scarcely be reproductive bodies. I interpret them as being simply abnormal extrusions of the ectoplasm (possibly produced by the pressure of the coverglass) analogous to the lobe-like pseudopodia which often appear after the trophozoites have been on the slide for several hours, and as utterly without any significance in the life-history of the species.

b. Preserved material. In sections of the urinary bladder trophozoites of the large form are often seen attached to the epithelial lining. Figure 61 represents a section through one of the younger, while figure 62 is from a section through a later stage. In the later stages there are always a much larger number of nuclei than in the smaller, disporous forms and these nuclei are of two very different types: viz., vegetative and generative.

The vegetative nuclei are much larger than the others from which they can be readily distinguished at a glance (figs. 61, 62, 65 to 67). They are filled with a well defined and evenly distributed reticulum of chromatin, and also contain at least one (rarely two) large rounded nucleolus which stains deeply with iron hematoxylin, but in strongly decolorized sections the interior is considerably lighter than the peripheral portion. In dried smears stained by Giemsa's method the vegetative nuclei stain very differently from the generative (fig. 65). In such preparations the chromatin of the generative nuclei take the purple stain as usual while the chromatin of the vegetative nuclei stain a light red and the nucleoli a light, uniform blue. The vegetative nuclei decolorize much more rapidly than the others in

acetone so that in strongly decolorized preparations they can scarcely be distinguished. Moreover, the vegetative nuclei are never surrounded by a specially differentiated area of cytoplasm as are the generative. The number of vegetative nuclei varies greatly, the older trophozoites containing more than the younger, although there are probably never less than four. Usually they are confined to the larger, attached end of the trophozoite where the cytoplasm stains more deeply and is evidently much denser than in other parts of the body.

The generative nuclei, although varying considerably in size, are always much smaller than the vegetative and have a very different appearance. They may occur singly or in groups and, like the generative nuclei of the disporous form, are surrounded by a specially differentiated area of cytoplasm forming a distinct cell (figs. 66 and 67). The chromatin is scattered in irregular clumps through the nucleus and there is usually no nucleolus. The generative cells divide mitotically and in a large trophozoite there may be a number of these cells present in the endoplasm. They all have a similar structure but may develop into two different types of reproductive bodies.

Occasionally a degenerative cell is seen, in which division of the nucleus is not followed by a corresponding division of the cytoplasm. Successive nuclear divisions follow in rapid succession until eight nuclei are formed, all enclosed in a common cytoplasmic mass (figs. 67 to 71). Meanwhile, the entire structure increases considerably in size, forming a very characteristic rounded body, sharply marked off from the surrounding endoplasm (figs. 59, 60, 69 and 70). These bodies are probably homologous with the pansporoblasts but have a very different history from the ordinary structures of that name. They are, in reality, similar to the gemmules formed by many species of Protozoa.

The gemmules at this stage have eight similar nuclei which are closely crowded together at the center. Surrounding them is a layer of cytoplasm which is more finely granular and stains more deeply than the undifferentiated endoplasm in which they are imbedded. Often, as in figures 69 and 70, a clear

space separates the gemmule from the surrounding endoplasm. This is no doubt due to shrinkage and indicates that the gemmule lies in a distinct cavity formed in the endoplasm of the mother trophozoite. In dried smears stained by the Giemsa method the cytoplasm of the gemmules stains a light blue while the cytoplasm of the mother trophozoite stains a light red. In the younger stages the nuclei of the gemmules contain no nucleoli, but by the time the 8-nucleate stage is reached there is usually a well defined nucleolus in each nucleus. There may be several gemmules present at the same time in a trophozoite as shown in figure 71 where there are nine, and trophozoites with even larger numbers are not rare.

The 8-nucleate state is usually as far as the gemmules develop within the mother trophozoite. They now make their way to the periphery (fig. 69), thence to the exterior by rupture of the ectoplasmic layer. I have several times observed the escape of gemmules from living trophozoites (fig. 60). Moreover, gemmules which have evidently just emerged from the mother trophozoites are common.

After the gemmules become free, four of the nuclei enlarge to form vegetative nuclei, while the other four become surrounded by a differentiated area of cytoplasm and develop into generative cells (figs. 72 and 73).

Occasionally the gemmules may be retained in the body of the mother trophozoite for a longer time. In such cases they increase in size and begin to develop in the same way as those which have become free (figs. 62, 74 and 75). The cytoplasm loses its dense, finely granular structure and no longer stains more deeply with plasma stains except near the center, where the denser material may persist for some time. Around the periphery the cytoplasm becomes vacuolated and exhibits a characteristic radiate structure (fig. 74). The nuclei increase in number by mitotic division and become differentiated into vegetative and generative as in the free forms.

This method of reproduction obviously furnishes a means for the rapid increase of the parasites within the host. Apparently the gemmules always develop into the large form of tropho-

zoites for I have never seen anything to indicate that they may develop into the smaller disporous type. During the months of June, July and August, when these observations were made, the formation of gemmules is certainly the principal method of reproduction in the case of the polysporous tropho-zoites, for sporulating individuals were always rare, and in many fish none at all were found, while trophozoites containing gemmules were always abundant. Whether this be true at other times of the year I am unable to say.

The spores are developed from generative cells similar to those which form the gemmules. Unlike the disporous form, the spores always develop in pairs from a common pansporoblast, the sporo-blasts of which are never separated. Each pansporoblast origi-nates from a single cell, but in this case the entire cell divides, so that the developing pansporoblast can be easily distinguished from the gemmules, in which only the nuclei divide. During the multiplication stage the generative cells separate soon after division, but in the pansporoblasts the daughter cells remain closely associated (figs. 76 and 77) although the outlines of the individual cells can usually be easily distinguished. Unlike the sporoblasts of the disporous form, the cytoplasm of the pan-sporoblasts does not ordinarily stain more deeply than the sur-rounding endoplasm. However, in a few cases I have noticed pansporoblasts in which the cytoplasm was denser and stained more intensely, while the outlines of the individual cells were indistinct and difficult to make out (fig. 79). Such pansporo-blasts appear strikingly like the sporoblasts of the disporous trophozoites except that the nuclei never contain a nucleolus.

The cells in the pansporoblasts do not have any such definite arrangement as in the sporoblasts of the disporous form, and it is, therefore, impossible to trace the derivation of the different cells of the spores, but there is no reason to think that it is essentially different. The fully developed pansporoblast contains twelve cells as in figure 78, in which eleven cells occurred in the section; the other cell which was in the next section is not shown. A care-ful study of a number of pansporoblasts which were sufficiently separated from adjoining cells to admit of an accurate count

has convinced me that there are never more than twelve nuclei present and that there is no trace of 'residual nuclei' at any stage. This is, of course, in accordance with the conditions found in the disporous forms.

The cells of the fully developed pansporoblasts are much alike except that the capsulogenous cells are larger and there is a deeply staining region in the cytoplasm at one side of the nucleus (fig. 78). The nuclei in these cells are also somewhat larger and the chromatin is less compact. Two of the nuclei shown at a lower level in the figure are larger and more flattened than the others and are probably in 'parietal cells' which form the valves of the spore membrane.

Figures 81 and 82 show later stages in the development of the spores which lie in a common cavity formerly occupied by the pansporoblast. Each spore is made up of six cells and here is no trace of 'residual nuclei' (figs. 81 to 83). In all essential respects they are like the spores of the disporous form (figs. 41 and 42, 80 to 82). Practically the only difference is in the capsulogenous cells which, in the polysporous forms contain, previous to the formation of the polar capsules, numbers of rounded granules staining deeply with the plasma stain. As previously stated I have been unable to distinguish any difference between the mature spores of the disporous and polysporous forms.

The process of mitotic division in the cells of the polysporous trophozoites is essentially the same as in the disporous, so that a detailed description is unnecessary. Figures 84 to 89 show the different stages of the process. The most striking difference is in the larger size of the chromosomes and the better development of the mitotic figure. As in the disporous form no sign of a centrosome was seen at any stage. I wish to emphasize the fact that while the chromosomes are much larger, the number is the same as in the disporous form. Moreover, as in the latter, the chromosomes are evidently in three pairs differing in size (figs. 86 to 87). One pair is considerably larger than the others; one pair is somewhat smaller; while the other pair is intermediate in size.

IV. GENERAL DISCUSSION AND REVIEW OF LITERATURE

Auerbach ('10) has recently given a very complete review of the literature on the Myxosporidia and it is, therefore, superfluous to go into a full discussion of it at this time.

1. Polymorphism

That the polysporous and disporous forms of trophozoites described in the preceding pages are one and the same species is, I believe, certain. At first I naturally took them for entirely distinct species, but as the work progressed it became more and more evident that only the assumption that both forms belong to the same species would account for the facts. In the first place the two forms are practically always found together. Moreover, the spores in both cases are practically identical, and this is, of course, the strongest evidence for considering the two types of trophozoites only different forms of the same species.[4] The development of the spore in the two types is very similar, differing only in unessential details. Also I would lay especial stress on the fact that although the chromosomes in the polysporous forms are much larger than in the disporous, the number and shape is the same in both cases. In both forms the chromosomes can be grouped in three pairs differing somewhat in size, the relative sizes of the chromosome pairs being the same. Furthermore, both types of trophozoites ingest the erythrocytes of the host, a phenomenon known to occur in only one other species of Myxosporidia. These two species form, so far as I know, the only examples of the ingestion of solid food in the entire group of the Sporozoa.

On the other hand I do not wish to minimize the fact that in appearance and structure the two types of trophozoites are unlike. In the examination of living material numerous examples of small trophozoites were seen which could not with certainty be assigned to either form. In cases where the ectoplasmic

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[4] It is well known that the structure of the spore is very constant in the Myxosporidia, each species having its own characteristic spore which shows little variation. So constant is the form of the spore that it is the principal character used in distinguishing between the different species.

processes are absent, the small polysporous trophozoites are often strikingly like the larger vegetative trophozoites of the disporous form. However, when stained there is no difficulty in distinguishing between the two types since as regards the nuclei they are very distinct. In a very few instances I have found individuals in which the nuclei were not typical for either type, and it may be that they were transition forms, but such trophozoites occurred so rarely in my preparations that I have been unable to satisfy myself as to their significance. Possibly transition forms occur only at certain seasons, but this must, for the present, remain pure conjecture.

Auerbach ('09 and '10) finds that Myxidium bergense forms both monosporous and polysporous trophozoites, but there is no such difference in the appearance of the two forms as in S. dimorpha. According to the same author (Auerbach '09a) Zschokella may be either monosporous or disporous, while Amerizew ('11) finds that the trophozoites of a species of Myxidium in the gall bladder of Cottus scorpius may form from one to three spores.

2. Multiplicative reproduction

Doflein ('98) has distinguished between multiplicative and propagative reproduction in the Myxosporidia. The former results in the increase of the organism within the host, or in other words in autoinfection, while the latter furnishes a means by which the parasite may pass from one host to another.

Plasmotomy is held by most writers to be the common method of multiplicative reproduction. However, in S. dimorpha, while plasmotomy has been observed in individuals after being removed from the bladder, there is no evidence that this is a common occurrence under normal conditions. In sections and Giemsa smears I have never seen multinucleate individuals which showed any evidence of having recently divided. In the disporous forms the character of the nuclei is such that plasmotomy, if it occur, should be easily detected; while the large numbers of this form which are practically always present indicate that some form of multiplicative reproduction is common,

I believe it must occur not later than the binucleate stage.   In
all later stages the nuclei are distinctly differentiated into vege-
tative and generative nuclei which are very characteristic, and
no trophozoites have been seen which could have been formed
by the division of such an individual.   On the other hand there
is evidence that the young binucleate forms may divide, and it
is interesting to note that such a method of reproduction has
been found by Auerbach in Myxidium bergense.   However, the
evidence of division is not conclusive, and for various reasons
this species furnishes very unfavorable material for the deter-
mination of this question.   I believe it is questionable whether
plasmotomy as defined by Doflein, occurs normally in the di-
sporous Myxosporidia.   The whole subject is in need of careful
investigation.

Turning to the polysporous form we find that here, also, there
is practically no evidence that plasmotomy is a normal occurrence.
It has been observed on the slide under abnormal conditions,
but there is little warrant for believing the process to occur
normally.   A few individuals were observed on the slide which
showed rounded processes strikingly like those described by
Cohn ('96) in Myxidium lieberkühni and which he believed to
be a process of budding.[5]   As previously stated, I believe these
processes are abnormal; formed as a result of the pressure of
the overlying coverglass, and that they have no significance in
connection with reproduction.

The normal method of multiplicative reproduction in the poly-
sporous form is, I believe, by the formation of gemmules.   Tro-
phozoites containing gemmules were so abundant as to leave
no room for doubt that multiplication by this method is rapid
and sufficient to account for the large number of polysporous
trophozoites found in many instances.

[5] Laveran and Mesnil ('02) deny that such budding occurs.   They find that
the trophozoites formed by plasmotomy may attach themselves to the larger
individuals, thus giving rise to the appearance erroneously interpreted by Cohn
as budding.   In S. dimorpha the exterior of the large trophozoites are distinctly
viscid and small trophozoites may become temporarily attached to them.   How-
ever, there is no doubt that the processes shown in figure 58 are due to extrusion
of the ectoplasm.

It would be strange indeed, if this method of multiplication is confined to the species under discussion. Erdmann ('11) has described peculiar protoplasmic bodies which sometimes emerge from disintegrating trophozoites of Chloromyxum leydigi, and which she considers 'vegetative reproductive bodies.' These bodies are apparently homologous with the gemmules of S. dimorpha, although Erdmann believes that their function is to provide for reproduction under unfavorable conditions only. This belief is apparently based on the fact that she observed their formation only in trophozoites growing on a gall agar culture medium, or introduced into the digestive tract of the host.

I have evidence (which will be reserved for a later paper) indicating that a similar method of endogenous budding, resulting in the formation of gemmules, occurs in a species of Ceratomyxa found in the gall bladder of a shark, Carcharhinus sp., and also in a Myxosporidian (probably a new genus) occurring in the urinary bladder of the flounder, Paralichthys albiguttus.

3. Propagative reproduction

Probably the sole method of propagative reproduction among the Myxosporidia is by the formation of resistant spores which can live for some time outside the body of the host. The process of sporulation, which is in all cases very complicated, has been studied by a number of investigators. Auerbach ('10) has given a very full review of the work on sporulation, and as the earlier accounts are so evidently erroneous in many respects, it is unnecessary to consider them here in detail. The most complete of the earlier accounts is that of Thélohan ('95), according to whom sporulation begins in Myxobolus by a single nucleus becoming surrounded by a spherical, condensed mass of protoplasm, which is in turn surrounded by a membrane. The nucleus then divides by mitosis until ten nuclei are present in the pansporoblast. The pansporoblast then divides into two parts (sporoblasts), each containing four nuclei. The remaining two nuclei remain outside the sporoblasts and gradually disappear. Each sporoblast becomes converted into a spore,

one nucleus passing into each of the two polar capsule cells while the other two nuclei remain in the sporoplasm.

In recent years a number of detailed studies of spore formation have appeared, notably those by Amerinzew ('09 and '11), Keysselitz ('08) and Schröder ('07 and '10). All these writers agree that six cells (eight in Chloromyxum) take part in the formation of the spore instead of four as held by earlier writers. Of these six, two form the valves of the spore membrane, two form the capsulogenous cells in which the polar capsules develop, and the remaining two the sporoplasm. With this conclusion my results are in perfect accord. There are, however, important differences in the history of the nuclei during sporulation. According to Amerinzew ('09) in Ceratomyxa drepanopsettae the trophozoite has at first two nuclei which are derived from the division of a single nucleus. Each nucleus then divides by mitosis into two nuclei of unequal size. The two smaller nuclei are vegetative, the two larger generative. The protoplasm becomes concentrated around each of the two generative nuclei to form cells inclosed in the endoplasm of the trophozoite. These two cells differ somewhat in size, the larger being a macrogametocyte, the smaller a microgametocyte. Each gametocyte then divides to form two gametes. Each gamete extrudes a quantity of chromatin into the cytoplasm after which each microgamete unites with a macrogamete, the single celled zygotes thus formed being the sporoblast from which the spores are developed later. In the formation of the spores each sporoblast divides into two cells differing in size. The smaller then divides again, giving a sporoblast with three cells, a larger which ultimately gives rise to two 'parietal cells' which form the valves of the spore membrane, and two smaller cells, one of which divides to form the two capsulogenous cells. The nucleus of the other small cell divides to form the bi-nucleate sporoplasm.

According to Schröder ('07) in Sphaeromyxa sabrazesi and Keysselitz ('08) in Myxobolus pfeifferi the conjugation of the gametes during sporulation takes place at a quite different stage. According to these writers the spores arise from 'propagative cells' which originate by generative nuclei of the plasmo-

dium becoming surrounded by a condensed mass of protoplasm. The propagative cells may multiply for a time by mitosis, but eventually proceed to spore formation. The propagative cell then divides into two cells of unequal size. Two such cell couples become associated and the two smaller cells form an envelope around the two larger which form the pansporoblast. The pansporoblast is thus from the first composed of two cells. Although the cytoplasm of these cells may fuse later, the nuclei remain distinct and divide repeatedly until the pansporoblast consists of twelve nuclei which may or may not be located in distinct cells inclosed within the envelope cells. The nuclei of the envelope cells, the so-called 'residual nuclei,' degenerate and take no active part in spore formation. The pansporoblast divides into two cell groups of six cells each, two cells in each group are parietal cells, two are capsulogenous cells, while the remaining two unite to form a binucleate cell which becomes the sporoplasm. Later, after the spore is fully formed, the two nuclei unite. Presumably in each sporozoite one nucleus is derived from each of the two original propagative cells but there is no direct evidence that this is the case. On the other hand, Mercier ('10) holds that in Myxobolus pfeifferi the pansporoblast is a zygote formed by the conjugation of a micro- and macrogamete. The single nucleus of the zygote then divides several times to form fourteen nuclei. Twelve of these separate into two groups, the sporoblasts, each containing six nuclei. Each sporoblast eventually forms a spore while the remaining two nuclei are thrown out and degenerate.

The principal discrepancy between the accounts of Amerinzew on the one hand, and Schröder and Keysselitz on the other is in regard to the time at which the gametes unite. According to Amerinzew copulation occurs previous to the formation of the sporoblasts while Keysselitz and Schröder hold that it is delayed until after the spores are formed. It is significant that in Myxobolus pfeifferi on which Keysselitz worked, Mercier found that copulation takes place previous to the formation of the pansporoblasts. This would bring the sporulation of the Polysporea in essential agreement with that of the Disporea.

On the other hand, in S. dimorpha, I have been unable to find any traces of sexual phenomena during sporulation in either the disporous or polysporous forms, although careful search has been made in both forms for evidence of a sexual process at this time. In the disporous form there is certainly nothing like the process described by Amerinzew in Ceratomyxa drepanopsetta. As previously described there can be little doubt that in S. dimorpha the sporoblasts are formed by the division of the nucleus of a uninucleate trophozoite, one of the daughter nuclei forming the vegetative nucleus, the other forming a 'propagative cell' which divides later to form the sporoblasts. Such trophozoites as are shown in figures 17 to 22 can, I believe, be explained in no other way. There is some evidence (which will be considered in detail later) that the original uninucleate cell is a zygote. If this be true, copulation must take place at a much earlier stage than in Ceratomyxa. It might be held that the binucleate trophozoite is formed by the fusion of two uninucleate trophozoites, although I have seen no evidence that this is the case. But in that event we would be forced to the remarkable conclusion that the nucleus of one gamete becomes the vegetative nucleus, while the nucleus of the other alone gives rise to the spores. At present I see no way of harmonizing my results with those of Amerinzew in C. drepanopsettae. However, in Myxidium sp. from the gall bladder of Cottus scorpius, according to Amerinzew ('11), a binucleate trophozoite may form sporoblasts directly without any previous sexual phenomena. In fact, judging from the short account which he gives of sporulation in this species, it differs from S. dimorpha only in relatively unimportant details.

In the polysporous forms sporulation in S. sabrazesi according to Schröder, and in M. pfeifferi according to Keysselitz, is in several respects quite different from the same process in S. dimorpha. In all three species the 'propagative cells' may multiply by mitosis. The daughter cells in S. dimorpha are, however, always of equal size and the pansporoblast originate from a single 'propagative cell'. There is no evidence of a preliminary fusion of cell couples as in S. sabrazesi and M. pfeifferi. In

S. dimorpha each pansporoblast eventually forms twelve—not fourteen—cells and a careful study of a large number of pansporoblasts has failed to show any trace of 'residual nuclei.' It seems strange that there should be such fundamental differences in the process of sporulation in forms so closely related to each other as the species under discussion, but in the present state of our knowledge it is impossible to harmonize the discordant accounts.

4. Mode of infection

I have no direct evidence of the method by which S. dimorpha gains entrance to a new host. The investigations of Auerbach ('09, '10) on Myxidium bergense and Erdmann ('11) on Chloromyxum leydigi have shown that, in all probability, the infection of new hosts takes place through the digestive tract. The sporozoite becomes free in the intestine and, as an amoebula, wanders actively up the bile duct to the gall bladder. Probably the infection of new hosts by S. dimorpha is accomplished in the same way, although the universal occurrence of the parasite in the urinary bladder of Cynoscion regalis is difficult to explain as the result of purely accidental infection.

There can be little doubt that the spores pass to the exterior soon after becoming free from the mother trophozoite, for in no case were large numbers of spores found in the bladder, although trophozoites in all stages of sporulation were abundant. Spores of S. dimorpha, when placed on the slide without previous exposure to sea water, and mixed with a drop of fluid from the pyloric caeca of the host, usually germinated within five to fifteen minutes. In most cases of germination the valves of the spore membrane separated along the sutural line followed by the emergence of the sporozoite which crept out, by an active amoeboid movement (fig. 47). In some cases the sporozoites were observed to disintegrate shortly after emergence (probably due to the action of the digestive fluid); others remained intact for the length of time they were under observation. In most cases, but not all, the filaments were extruded from the polar capsules on the addition of the caecal fluid. A few spores

failed to germinate or extrude their polar filaments after being exposed to the caecal fluid for three hours, when the experiment was discontinued.

It appears probable, therefore, that the free spores, when taken into the intestine of the host, germinate, and the sporozoites, as free amoebulae, actively make their way into the urinary bladder. Whether the amoebulae then go through an intercellular stage as has been found by a number of investigators to be the case in other species, it is impossible to say. While I have noted a number of instances in which the epithelial cells of the bladder contained an intracellular parasite I am not at all certain that the parasite was a stage of S. dimorpha. The urinary bladder and Wolffian ducts of C. regalis are often infected with a species of Leptotheca in addition to S. dimorpha, and while there is no difficulty in distinguishing the free forms of the two species, owing to their very different structure (especially as regards the nuclei), I am by no means certain that it is equally easy to distinguish the intracellular forms. Certainly the nuclei of the intracellular parasites are quite different from those of the free-living trophozoites of S. dimorpha.

5. Fertilization

Finally comes the question: Is there any evidence of a sexual process in the life-history of S. dimorpha? We have already seen that no sexual phenomena are associated with sporulation in this species, so that if there be a sexual process, it must occur at some other stage. Although the two nuclei of the sporoplasm are still distinct in the fully matured spore, it is probable that they fuse later (possibly at the time of germination), as has been found to be the case in so many species of Myxosporidia. Such a fusion would be a case of endogamy, but there is some evidence that exogamy also occurs.

In Giemsa smears there are occasionally seen small mononuclear cells which are quite different in appearance from the mononuclear trophozoites shown in figures 8 and 9. Not only are they much smaller, but the amount of cytoplasm is relatively

much less and appears perfectly homogeneous, never granular as in the ordinary mononuclear trophozoite (fig. 3). Occasionally such cells lie side by side while similar cells containing two nuclei are not uncommon (figs. 4 and 5). The appearance of these binucleate cells strongly suggests that they are formed by the fusion of two cells like that shown in figure 3, while figure 6 may plausibly be interpreted as a somewhat later stage in which the nuclei are partially fused, and figure 7 may represent a later stage after complete fusion of the nuclei. If the above interpretation be correct figure 7 represents the earliest stage of the definitive trophozoite, formed by the fusion of two similar gametes, in which the cytoplasm has not yet developed the granular structure so characteristic of the later stages.

On the other hand, were it not for figure 6, the cells shown in figures 3 to 7 might just as plausibly be interpreted as different stages in the division of a cell like figure 7, in which case the sequence of the different stages would be just the reverse of that suggested above. Although I have devoted much time to the study of these stages, I am still unable to decide which is the correct interpretation. The cell shown in figure 6 is the only case I have found where the nuclei appeared to be fusing. On the other hand I have found no case in which the nuclei appeared to be undergoing division in cells characterized by a small amount of homogeneous cytoplasm. Several cases of nuclear division in the larger mononuclear trophozoites have been observed, but they can always be distinguished by the relatively larger amount of granular cytoplasm.

For the present, then, it must be considered doubtful whether the stages shown in figures 3 to 7 represent a case of copulation or simply a method of multiplication by division of small mononuclear trophozoites.

V. SUMMARY

1) The urinary bladder and Wolffian ducts of the squeteague, Cynoscion regalis, contain large numbers of a myxosporidian parasite, Sphaerospora dimorpha, n. sp.

2) This parasite occurs in two very distinct forms, one of which is disporous, the other polysporous.

3) Both disporous and polysporous forms ingest solid food.

4) The spores produced by each form are identical and the number and form of the chromosomes are the same in both cases.

5) In both disporous and polysporous forms the trophozoites contain both vegetative and generative nuclei.

6) In the disporous form the generative or 'propagative' cell forms two sporoblasts, each containing six cells, two of which form the sporoplasm, two the capsulogenous cells, while the other two form the spore membrane.

7) In the polysporous form the generative or 'propagative' cells may form either gemmules or pansporoblasts.

8) Sporulation in the polysporous form is essentially the same as in the disporous, differing only in unimportant details.

9) There is no evidence of a sexual process connected with sporulation in either form.

10) In all probability the only method of multiplicative reproduction in the polysporous forms is by the formation of gemmules.

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PLATE 1

All figures, with the exception of figure 47, were drawn with the aid of the camera lucida at the level of the base of the microscope. The magnification, where not otherwise specified, is 1550 diameters. All figures, where not otherwise noted, were drawn from sections of the urinary bladder fixed in formol-corrosive-acetic and stained with iron hematoxylin and congo red.

Figures 1 to 55 are of the disporous, figures 56 to 89 of the polysporous form.

1 Living vegetative trophozoite shortly after being placed on the slide. × 1400.

2 Living vegetative trophozoite with two erythrocytes in the endoplasm. × 1400.

3 Small mononuclear cell the nucleus of which is surrounded by a thin layer of homogeneous cytoplasm. Giemsa smear.

4 and 5 Cells with two nuclei but otherwise similar to figure 3. Giemsa smear.

6 Cell with homogeneous cytoplasm and two nuclei which appear to be fusing. Giemsa smear.

7 Mononuclear trophozoite with homogeneous cytoplasm which is not as abundant as is usually the case at this stage. Giemsa smear.

8 Mononuclear trophozoite in which the cytoplasm is more abundant than in figure 7 and is also distinctly granular. Giemsa smear.

9 Mononuclear trophozoite similar to figure 8. At one side can be seen the pseudopodia by means of which it was attached to the urinary epithelium.

10 to 12 Binuclear trophozoites larger than figures 4 and 5 and with granular cytoplasm.

13 to 15 Binuclear trophozoites in which each nucleus is plainly surrounded by a specially differentiated area of the endoplasm. Figure 13 from a Giemsa smear.

16 Trophozoite with three nuclei of approximately same size. No specially differentiated area of endoplasm could be distinguished around the nuclei.

17 Trophozoite with three nuclei one of which is considerably larger than the others. Giemsa smear.

18 Similar to figure 17 except that a specially differentiated area of endoplasm could be distinguished around each of the smaller nucler. Giemsa smear.

19 Similar to figure 18 except that the structure of the larger (vegetative) nucleus is plainly unlike that of the other two. Giemsa smear.

20 Two trophozoites, one with only three, the other with four nuclei. Giemsa smear.

21 Two trophozoites, one with three, the other with four nuclei. In each trophozoite the vegetative nucleus can be easily distinguished. At the right can be seen the pseudopodia by means of which they were attached to the urinary epithelium.

22 Trophozoite with four nuclei. The three generative nuclei are inclosed in distinct cells.

23 Trophozoite with five nuclei. Three of the four generative nuclei are inclosed in distinct cells, which can be made out only with difficulty. No definite sporoblasts can be distinguished.
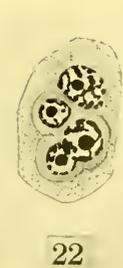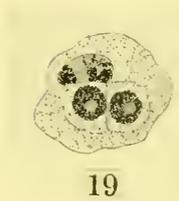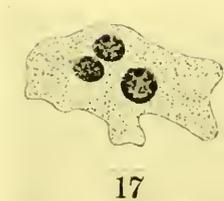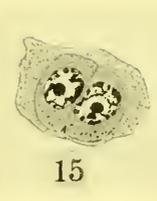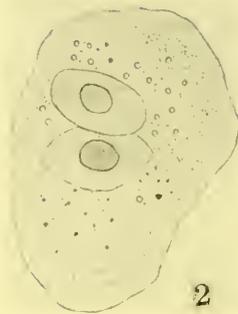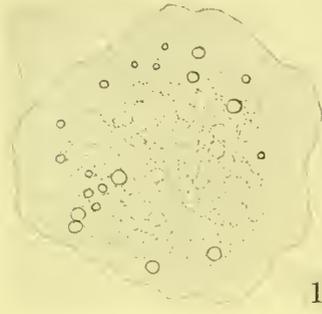
PLATE 2

24   Trophozoite with one vegetative and four generative nuclei, the latter associated in pairs to form the sporoblasts. Giemsa smear.

25   Trophozoite containing one vegetative nucleus and two sporoblasts, one with two, the other with three nuclei. Giemsa smear.

26   Trophozoite in which the sporoblast has shrunken away from the surrounding endoplasm. Only two of the three nuclei of the sporoblast are in the section.

27   Trophozoite showing one entire sporoblast composed of three cells. Only part of the other sporoblast occurs in the section.

28   Trophozoite containing two sporoblasts, each with three nuclei but no distinct cells could be made out. One vegetative nucleus present. All the nuclei in the trophozoite are shown.

29   Trophozoite with one four-celled and one three-celled sporoblast. Only one vegetative nucleus present.

30   Trophozoite in which the vegetative nucleus is dividing amitotically. Only one sporoblast with five nuclei occurs in the section.

31   Trophozoite with one three-celled and one five-celled sporoblast. Only one vegetative nucleus present.

32   Three-celled sporoblast drawn under a higher magnification. Note that one cell is distinctly larger than the other two. × 2000.

33   Three-celled sporoblast, in which the two smaller cells are dividing mitotically. × 2000.

34   Sporoblast in which one of the smaller cells has just divided while the other is in a late anaphase. × 2000.

35 and 36   Five-celled sporoblasts formed by the division of the two smaller cells of the three-celled sporoblasts. × 2000.

37 and 38   Five-celled sporoblasts in which the large end cells are preparing to divide amitotically. Note that in figure 38 the nucleolus shows a median constriction and that in figure 37 there are two nucleoli. × 2000.

cap.c., capsulogenous cell.   *par.c.*, parietal cell.
gem., gemmule.   *sp.c.*, sporoplasm cell.
g.c., generative cell.   *v.n.*, vegetative nucleus.
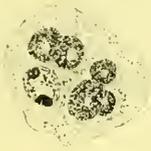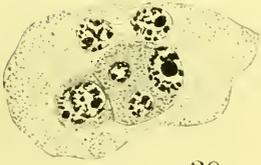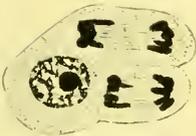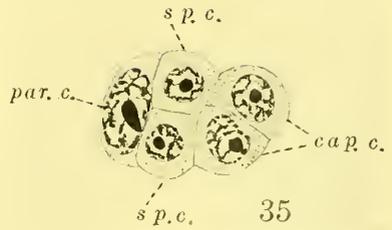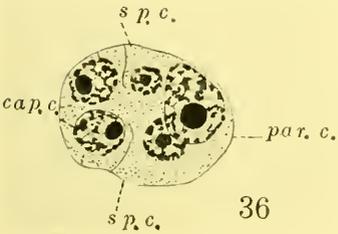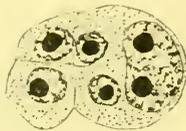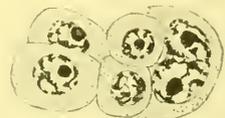
PLATE 3

39 and 40 Optical sections through the same trophozoite showing all the nuclei. There are twelve cells in the sporoblasts becoming arranged to form the spores. Only one vegetative nucleus is present. $\times$ 2000.

41 Trophozoite in which the cells have become definitely arranged to form the spores. All the nuclei are shown. The parietal cells surround the capsulogenous and sporoplasm cells. The capsulogenous cells can be easily distinguished by their larger nuclei.

42 Trophozoite in which the spores are slightly more advanced than in figure 41. Only one spore is shown. A deeply staining body is present in the cytoplasm of the capsulogenous cells.

43 Nearly mature spore. Only one capsulogenous cell is shown.

44 Living trophozoite containing two mature spores. $\times$ 640.

45 Mature spore as it appears when set free in the urine. $\times$ 1400.

46 Mature spore slightly compressed under the cover-glass. $\times$ 1400.

47 Free-hand drawing of germinating spore.

48 to 55 Different stages in the mitotic division of the sporoblast cells.

48 and 49 Prophase. $\times$ 2650.

50 to 52 Polar views of the equatorial plate showing six chromosomes. $\times$ 2650.

53 and 54 Anaphase. $\times$ 2650.

55 Telophase. $\times$ 2650.

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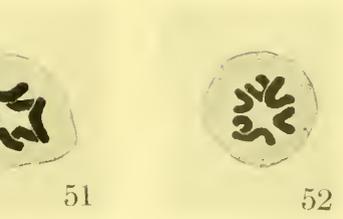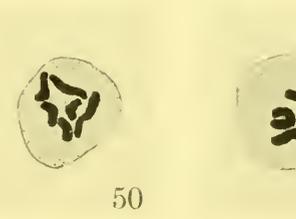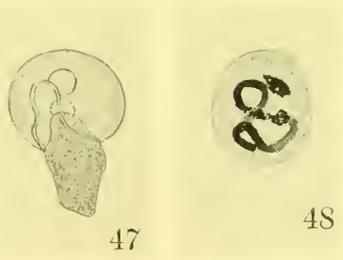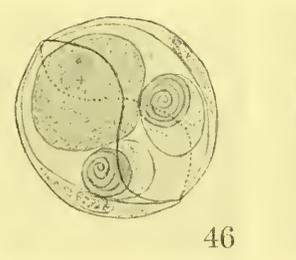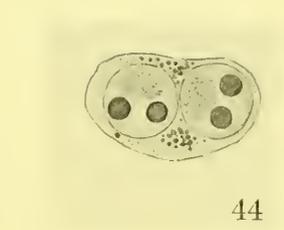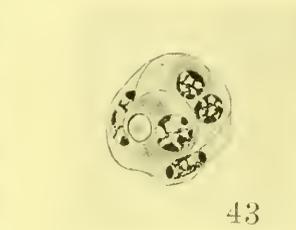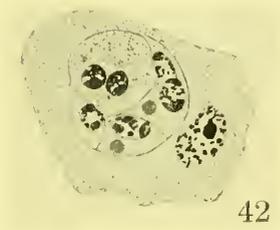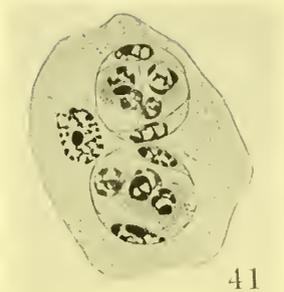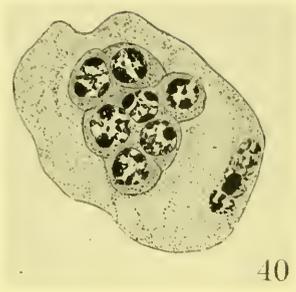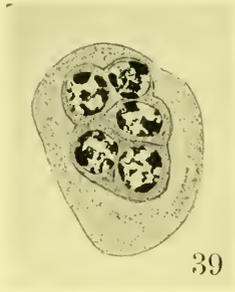
56   Living trophozoite of the polysporous form shortly after being placed on the slide.   × 640.

57   Small living trophozoite of the polysporous form containing several erythrocytes in different stages of disintegration.   × 640.

58   Living trophozoite with a number of bud-like ectoplasmic processes. × 640.

59   Living trophozoite with a number of gemmules in the endoplasm.   × 640.

60   Living trophozoite from which a gemmule is just escaping.   × 640.

63   Section through base of young trophozoite showing protoplasmic processes by which it is attached to the urinary epithelium.

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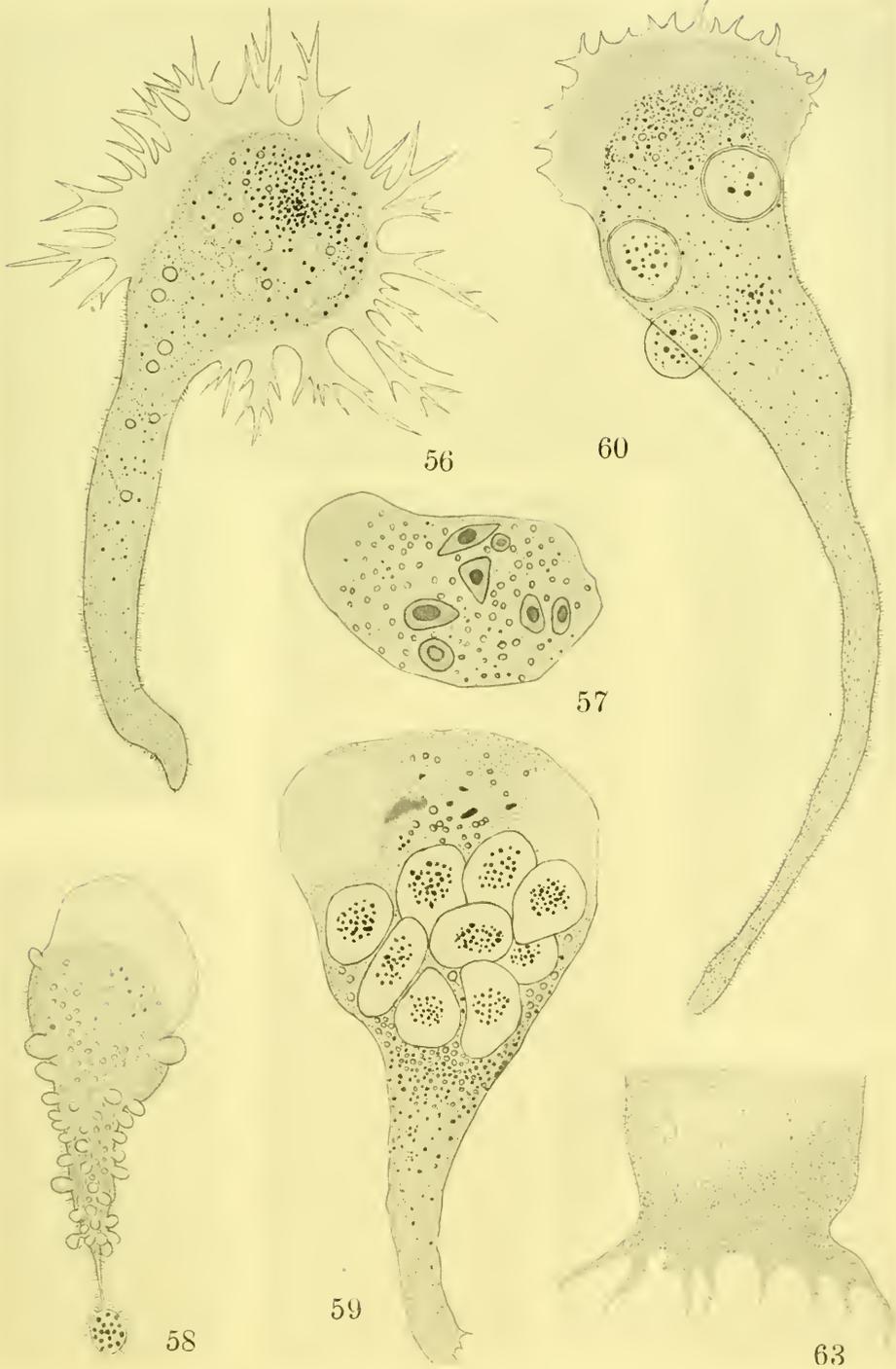
61   Section through trophozoite attached to lining of the urinary bladder. × 525.

62   Section through older trophozoite than in figure 61.   × 525.

64   Section through the base of an older trophozoite showing organs of attachment more highly developed.

65   Portion of a trophozoite from a dried smear showing vegetative and generative nuclei.   Two of the generative nuclei are dividing mitotically.   × 1400.

66   Portion of section through trophozoite showing generative cells and vegetative nuclei.

68   Section through small part of trophozoite containing a gemmule with only four nuclei.

69   Section through gemmule containing eight nuclei only five of which are shown.   The cavity in which the gemmule lies can be distinctly seen.

70   Section through gemmule at periphery of trophozoite and apparently just ready to emerge.   One nucleus is dividing mitotically.   Only part of the nuclei are shown.   At one side the gemmule is shrunken away from the surrounding endoplasm.

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_g.n._

_v.n._

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_v.n._

_g.c._
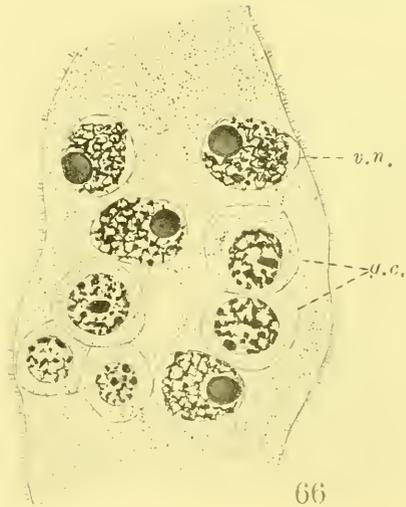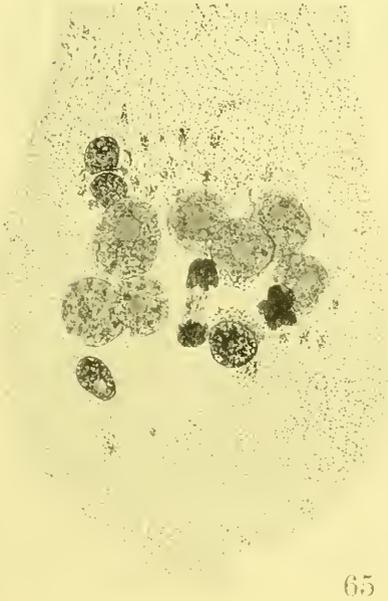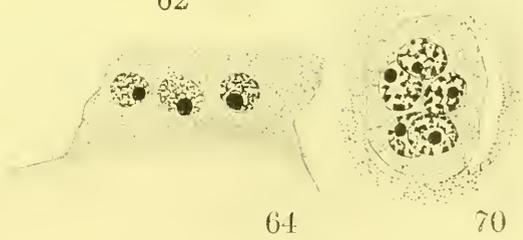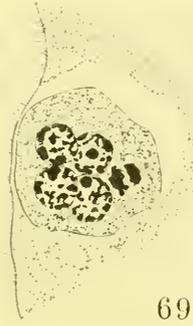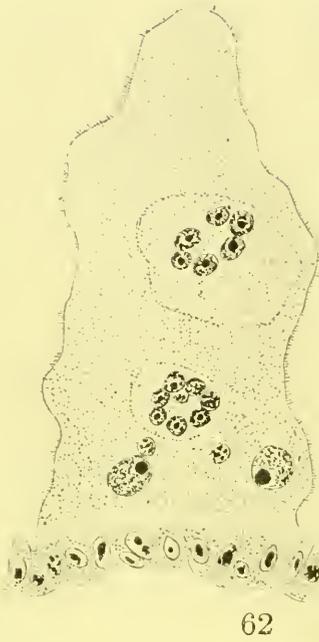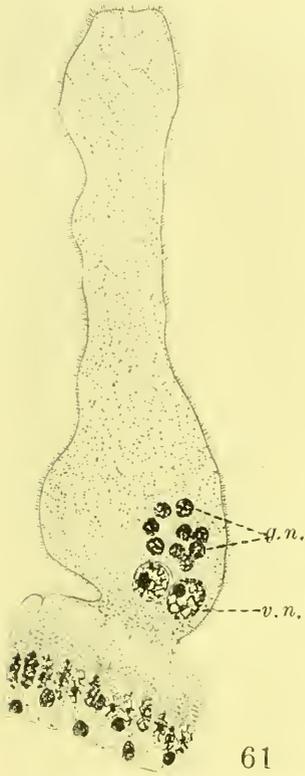
PLATE 6

EXPLANATION OF FIGURES

67 Section through small portion of trophozoite showing a number of generative cells, two gemmules and one vegetative nucleus.

71 Portion of trophozoite showing a number of gemmules. Drawn from a dried smear stained by Giemsa's method. $\times$ 1400.

72 and 73 Gemmules which have recently emerged from the mother trophozoite. Note that four of the nuclei are becoming enlarged to form vegetative nuclei.

74 Section through gemmule which has been retained in the mother trophozoite longer than usual. The nuclei are already differentiated into vegetative and generative nuclei, the latter being inclosed in distinct cells.

75 Similar to figure 74 except that the gemmule is in a somewhat later stage of development.

76 Portion of trophozoite showing several pansporoblasts. All the nuclei are inclosed in distinct cells. Only part of the cells are shown.
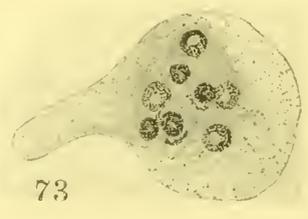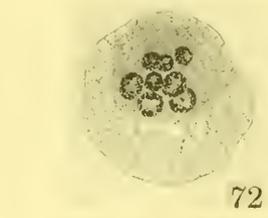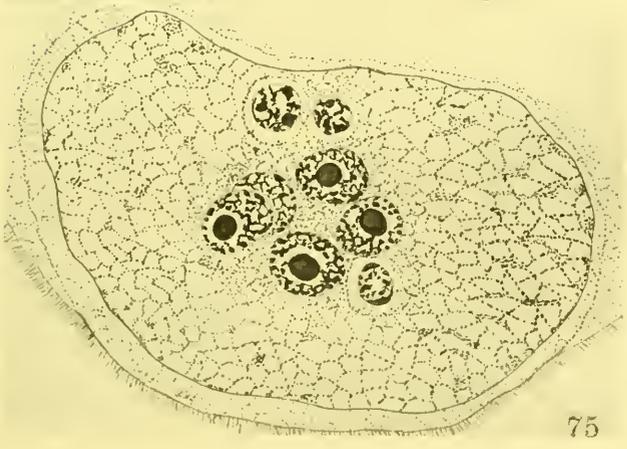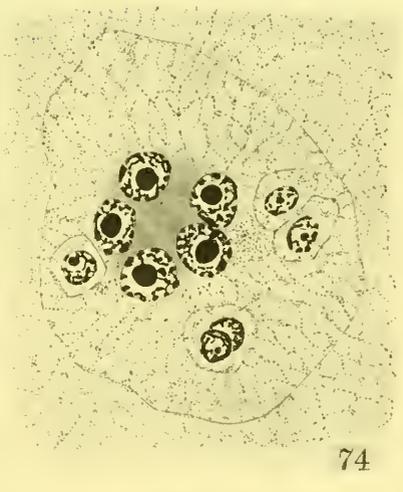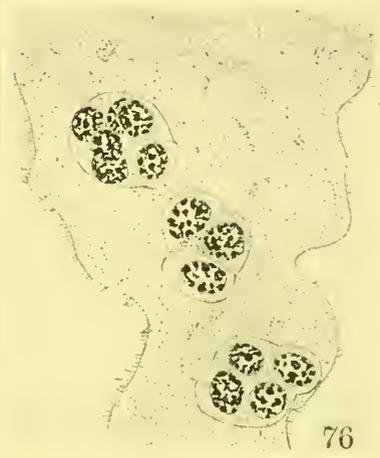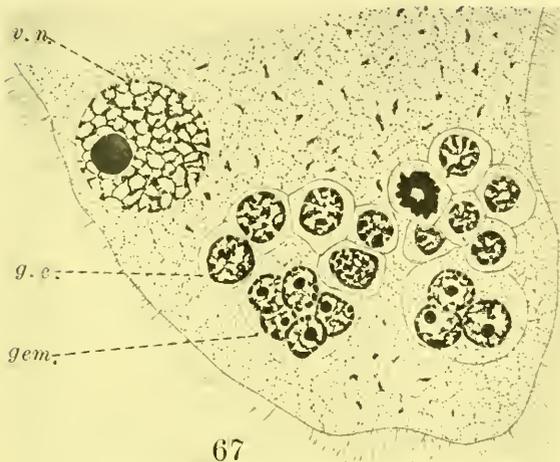
77   Portion of a trophozoite showing parts of several pansporoblasts and one gemmule.

78   Portion of trophozoite containing a pansporoblast.   There are only eleven nuclei in the section the twelfth being in the adjoining section.

79   Portion of trophozoite containing parts of two pansporoblasts.   Note that the cytoplasm of the pansporoblasts stain deeply while the cell outlines are very difficult to distinguish.

80   Nearly mature spore.   All the nuclei in the spore are shown.

81 and 82   Each figure shows two spores formed from the same pansporoblast. Slightly later stage than in figure 80.   All the nuclei are shown.

83   Portion of sporulating trophozoite in which the cells of the pansporoblast have become definitely arranged to form spores.   Drawn from a dried smear stained by Giemsa's method.   × 1400.

84 to 89   Different stages in sporoblast cells of the polysporous form.

84   Prophase.   × 2650.

85   Side view of metaphase.   × 2650.

86 and 87   Polar views of equatorial plates showing six chromosomes in each case.   × 2650.

88   Anaphase.   × 2650.

89   Telophase.   × 2650.
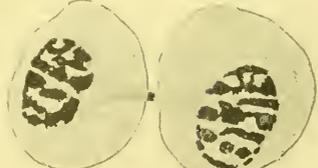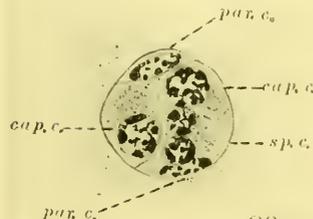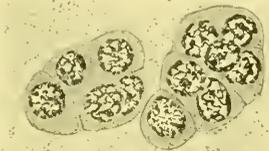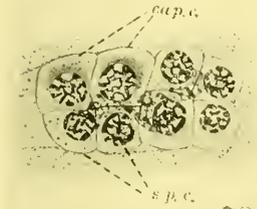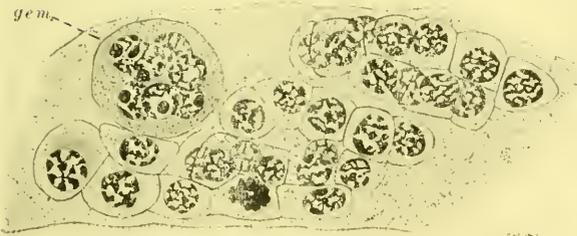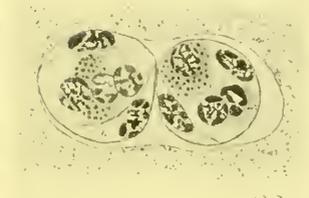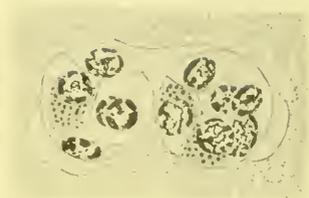
FORM AND GROWTH IN FISHES

SELIG HECHT

ELEVEN FIGURES

CONTENTS

INTRODUCTION

Organic growth has been defined as an increase in the volume of the living material (Morgan, '07, p. 240). And yet, curiously enough, in practically all the work that has been done on the growth of organisms, the *weight* has been the main consideration (Minot, '08, and Kellicott, '08). The usefulness of an organ and the adaptedness of an organism to its environment are hardly functions of their weight. The form, however, is of prime importance. The data which have been accumulated in the past give practically no information with regard to the relation of form to the rate of growth.

It has been convenient to study this matter in fishes by correlating weight, form, and rate of growth, not only of the entire animal, but of several morphologic divisions of its surface. In an earlier paper Crozier and the writer ('14) established that in the weakfish, Cynocion regalis, weight, length, width, depth, etc., were closely related to one another, and that these relations could be expressed by simple mathematical equations. After a preliminary study of the relation of weight to length in the

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smooth dogfish, Mustelus canis, I was convinced that in this species the form of an individual remained constant throughout its life, and that here, too, weight was a function of form.

It seemed therefore desirable to attempt a more extensive consideration of these relationships as found in fishes. This I have done. Other matters, which came up in the course of the investigation, have an important bearing on the theoretical interest of the subject, and will be dealt with below.

MATERIAL AND METHODS

The selection of species for study was dependent on their abundance, and on the range of size that they offered. During July and August of 1913, at Beaufort, N. C., six species were found which fulfilled these requirements sufficiently well. As was to be expected, a random catch was composed mainly of medium-sized individuals. In order, therefore, to have a series covering as large a range as possible, the specimens were, in a measure, selected according to length, so as to give approximately equal numbers of all the lengths represented. Table 1 gives the family, specific, and common names, and also the number of individuals of each species used in this study.

TABLE 1

FAMILY	SPECIES	NO. OF SPEC'MENS MEASURED
Clupeidae (Herrings)	Brevoortia tyrannus (Menhaden)	90
Engraulidae (Anchovies)	Anchovia brownii	158
	Anchovia mitchilli	63
Stromateidae (Butter-fishes)	Peprilus alepidotus (Butterfish)	54
Haemulidae (Grunts)	Orthopristis chrysopterus (Pig-fish)	168
Scianidae (Drums)	Leiostomus xanthurus (Spot)	90

The weights were taken on a platform balance reading directly to 0.1 gram. More accurate weighings were unnecessary. All of the measurements, except those of length in the case of the larger fish, were made with dividers. For the determination of the length of the larger specimens, the fish were placed on a

centimeter scale and the length read off directly.   Measurements
were always read to 0.5 mm.   The same scale and balance were
used throughout the work.

Such sources of error as evaporation and post-mortem shrink-
age were avoided by weighing and measuring the material imme-
diately on its arrival at the laboratory.   The surface water and
mucus were removed before weighing.   After a preliminary in-
vestigation, carried out on about half the number of individuals
used in this work, it was found unnecessary to apply a correction
for the weight of the stomach contents.

The sources of variation occasioned by the physiologic dis-
turbances of spawning (Miescher, '81, and Greene, '14) is, in
this case, negligible.   None of the specimens showed any pro-
nounced development of the gonads, and in many cases it was
difficult to determine sex because of the immature or unripe
conditions of the sex organs.

<center>WEIGHT AND LENGTH</center>

The data obtained are given in part in figures 1 to 5.   In all
cases weight is given as the ordinate and length as the abscissa.
The circlets represent single determinations in most cases, al-
though there are many duplicates and even triplicates.   The
individuals of each species show a clear correlation between the
weight and the length.   Since the circlets represent males and
females in about equal numbers, and since these are well dis-
tributed about the smoothed curve, we may conclude that sex
does not influence the constant relation between weight and
length.

This agrees with what has been shown to be true for the
weakfish (Crozier and Hecht, '14) and for the dogfish (Hecht, '13),
also with Heincke's results on the European plaice.   Donaldson
('12) states that in the albino rat, differences in sex are negli-
gible in the relation of total weight to length as well as to weight
of brain and of spinal cord.   Although not explicitly stated, the
figures given by him (Donaldson, '98) for the frog, lead to the
same conclusion.

The curves shown in the figures (1 to 5) are all of the form

$$y = ax^3$$

where y is the weight, x is the length, and a is a factor which varies with the species and with the units used. Table 2 gives the values of a for the six species studied by me and for five other
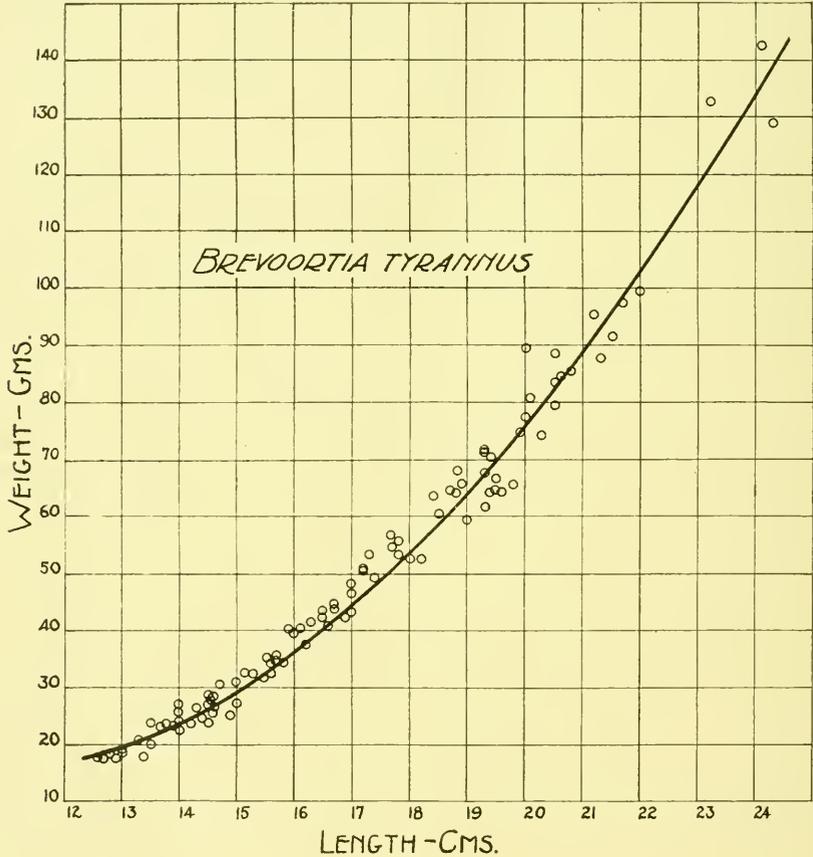
Figure 1

species. The figures for the plaice (Pleuronectes platessa) represent an average taken from the values given by Johnstone ('12). The values for the European herrings, Clupea sprattus and C. harengus, I calculated from data given by Jenkins ('02), which he used for an entirely different purpose.

TABLE 2

Weight-length constant

SPECIES	$a \times 10^2$
Mustelus canis..	0.274
Brevoortia tyrannus..	0.912
Clupea sprattus...	0.695
Clupea harengus..	0.631
Anchovia brownii...	0.709
Anchovia mitchilli..	0.618
Peprilus alepidotus..	1.70
Cynoscion regalis...	0.877
Leiostomus xanthurus..	1.15
Orthopristis chrysopterus......................................	1.30
Pleuronectes platessa..	1.07

In 1899 Hensen, at the suggestion of Reibisch ('99), and with the latter's data, divided the weight of a series of plaice by the cube of the length, in an attempt to get some weight-length
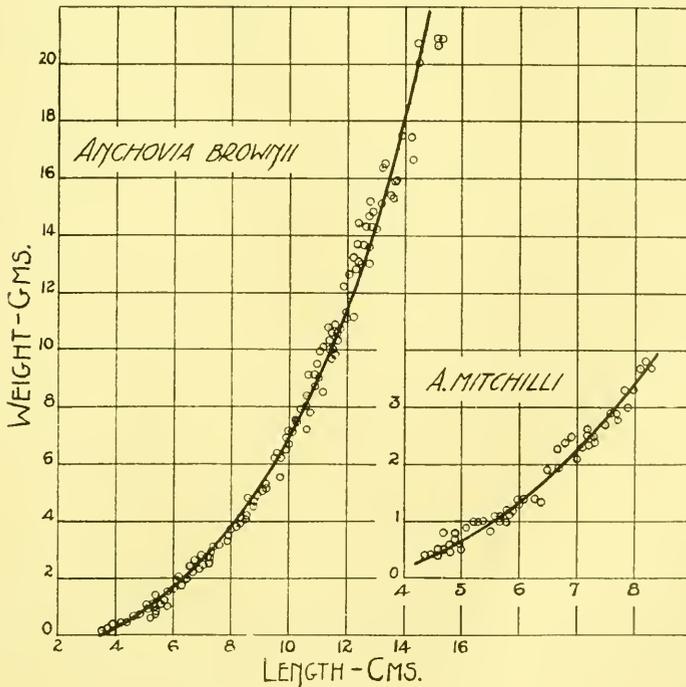
Figure 2

relation. For different series of individuals he got different re-
sults, and because of the small number of fishes studied, arrived
at no definite conclusions. But in the light of the later work of
Heincke et al. ('07) and of Johnstone ('14), his variations are
easily explainable.
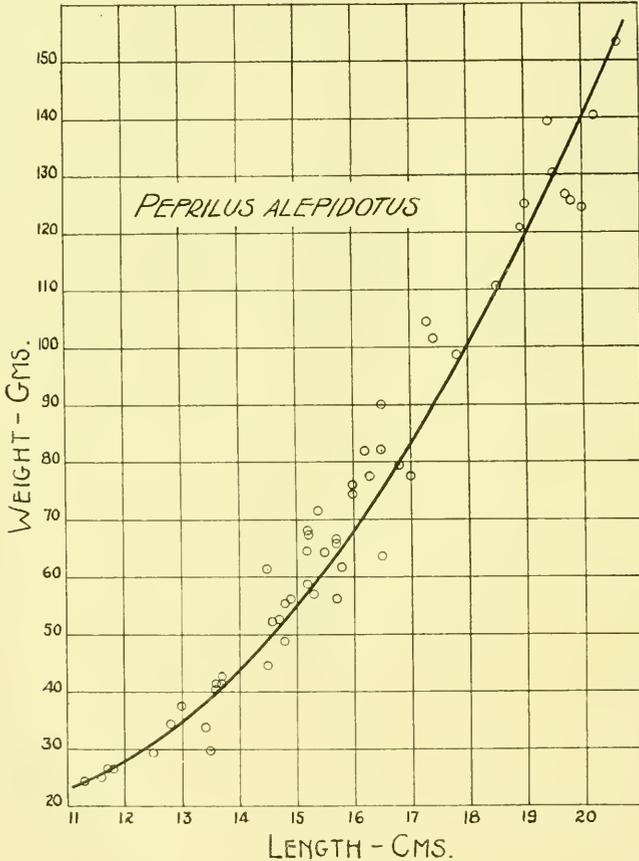
Figure 3

Working with the same species, Meek ('03) found, upon
plotting a large number of cases, that weight is a function of the
cube of the length. Apparently unaware of this work, Fulton
('04) tested the same idea on several species of fish. He found
that weight above a certain point increases at a greater rate than

the cube of the length.[1]   Meek ('05), continuing his work, showed that the weight-length relation which he had established for the plaice was also true for the sole, the dab, the flounder, and the turbot.   At the suggestion of D'Arcy Thompson, Heincke and his co-workers adopted this cube relationship and used it

ORTHOPRISTIS CHRYSOPTERUS
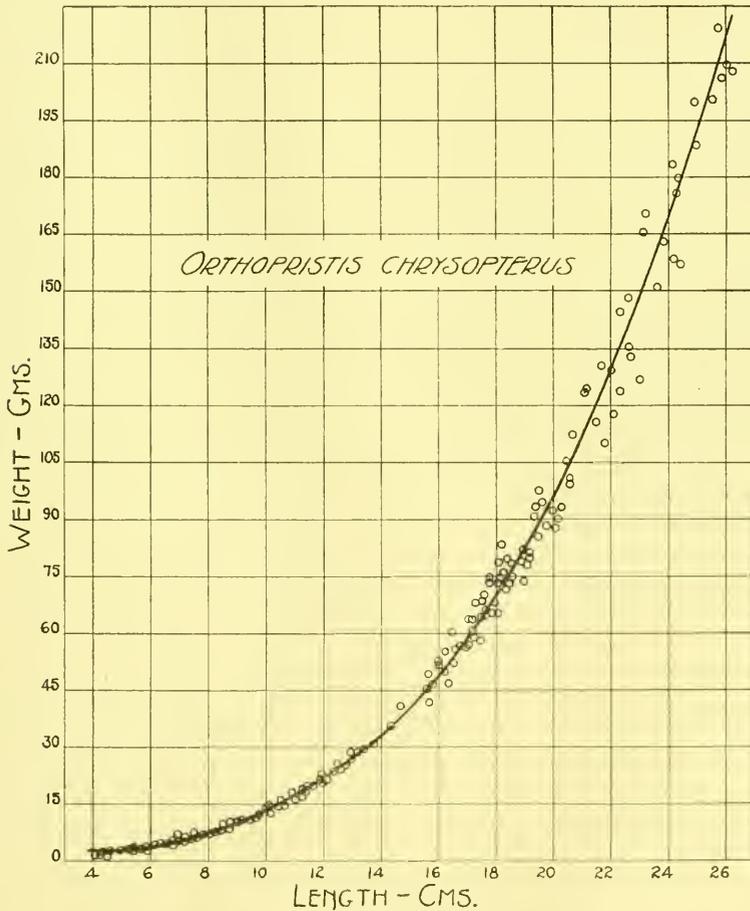
Figure 4

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[1] I, too, have found this to be the case with one or two species.   The increase, although very regular, is, however, well within the limits of variation, and if an average be taken of the values of the coefficient at different parts of the curve, the variation produces no larger error than that found in the species which conform to the simpler cube relationship.

in their work on the plaice. Since that time, weight-length coefficient has been used mainly in the study of the European plaice by Johnstone ('14) and others.[2]

Thus, at least eleven different species representing seven distinct families, have been investigated for this relation. It seems therefore safe to conclude that in fishes generally the correlation of weight to length is such that the weight is equal to a constant factor times the cube of the length:—

$$weight = a \times (length)^3.$$

It is to be expected that there will be a seasonal variation in the value of this coefficient corresponding to the cyclic physiological changes that the species undergoes in the matter of nutrition and reproduction. Indeed, Johnstone ('11) states that the value of a varies during the different seasons of the year, and furthermore, that this coefficient is the only convenient index of the 'condition' of the plaice inhabiting any given fishing ground. When the plaice is well nourished or sexually ripe, the value of a is large, whereas, when the fish is ill nourished or spent, the value of the coefficient is low. Generally speaking, it is greatest in the summer and least in the winter (especially, Johnstone, '14, fig. 5).

It was this variation that caused the failure of Hensen and Reibisch in their early attempts to get at this constant.

BODY MEASUREMENTS

These results suggest (Hecht, '13) that the form of an individual fish is constant, within rather narrow limits, throughout its life (Spencer, '71, p. 123). To study this in detail, I examined the relations among the morphologic surface divisions of each species. Since the relation of weight to length is known and is constant, length was chosen as the unit of comparison among the parts studied, viz., the length of head, body, and tail, and the width and depth of the animal.

The head-length in all species means the distance from the tip of the snout to the end of the opercular bone. The body-

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[2] When Crozier and I found the same relation for the weakfish, we were entirely unaware of this European work on the plaice. It was only after the present work was completed that these contributions came to my attention.

length is the distance from the tip of the opercular bone to a
point on the lateral line immediately below, or above, the end
of the most posterior median fin. In some species this is the
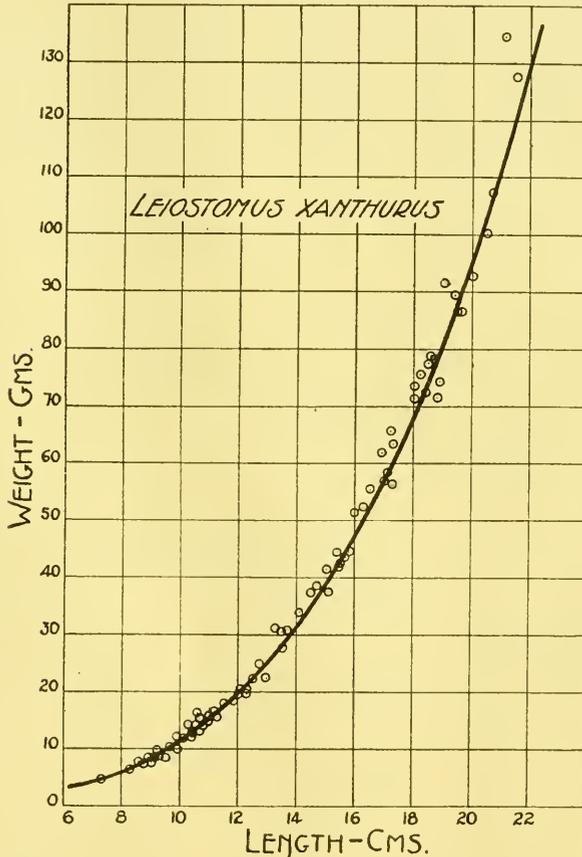
Figure 5

dorsal fin (e.g., Leiostomus xanthurus); in others it is the anal
fin (e.g., Brevoortia tyrannus). The tail-length is the distance
from the end of the body-length to the tip of the caudal fin
projected on to the long axis.[3] The width is the distance per-

[3] For a figure showing the exact location of the points serving for these meas-
urements, the reader is referred to figure 3 in the paper by Crozier and Hecht.
See also figure 11 of the present paper.

pendicular to the sagittal plane at the widest part of the fish, anterior to or dorsal to the stomach region. This place was chosen for measurement to avoid any error which might arise as a result of the distention by the stomach contents. The depth is approximately the greatest depth. It is the distance from the origin of either the anal or the dorsal fin to the other edge of the fish, on a line perpendicular to the long axis of the body. In Brevoortia tyrannus and both species of Anchovia the origin
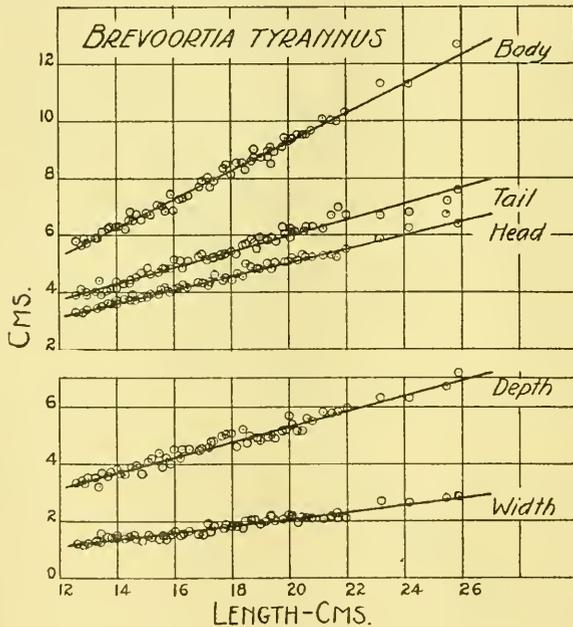
Figure 6

of the dorsal fin was used, whereas in the remaining species the origin of the anal fin was used as the starting point. Thus no artificial distinctions were made, and each part represents very nearly what it signifies in ordinary usage.

Figures 6 to 10 give the data obtained. What was said in reference to the plotting of the points in the previous figures (p. 381) applies here. The smoothed 'curves' passing through the points are, in all cases, straight lines, indicating that the relations among the various parts may be expressed by simple

formulae. Using length as the basis of comparison, each straight line is, in general, represented by the equation

$$y = cx + b$$

in which y is the dimension of the body part (head, depth, etc.), x is the length, while c is the 'slope' or 'tangent' of the line, and b is the distance from the origin of the axes of coordinates to the point where the line crosses the axis of ordinates. In all of the lines given in these figures, b is practically zero, since the lines all converge and meet at a point approximately (0,0). The equation then becomes

$$y = cx.$$

In table 3 the values of c are given for each of the five measurements for each species. Cynoscion regalis is included for comparison.

TABLE 3

Values of the constant c

SPECIES	BODY	TAIL	HEAD	DEPTH	WIDTH
Brevoortia tyrannus............	0.458	0.300	0.253	0.263	0.103
Anchovia brownii..............	0.473	0.325	0.223	0.153	0.084
Anchovia mitchilli.............	0.526	0.288	0.205	0.161	0.081
Peprilus alepidotus............	0.477	0.327	0.204	0.470	0.103
Cynoscion regalis..............	0.530	0.273	0.215	0.135	0.115
Leiostomus xanthurus.........	0.478	0.299	0.241	0.231	0.106
Orthopristis chrysopterus......	0.424	0.334	0.266	0.250	0.111
Average.......	0.481	0.306	0.229		0.100

The only reference to this sort of relation with which I am acquainted is by Meek ('05), who showed that width and length bear this simple straight-line relation to each other in the dab, flounder, sole, and turbot.

BODY MEASUREMENTS AND WEIGHT

An attempt was made by Heincke et al. ('07) to find a relation between body measurements and weight. They chose length and depth, and found that

$$g = kl^2 d$$

where g is the weight, l is the length, d is the depth, and k is a constant. Independently of this Crozier and Hecht ('14, p. 145) found a more general relation between body measurements and weight. Since depth and width are each linear functions of the length, the values of depth and width divided by their respective
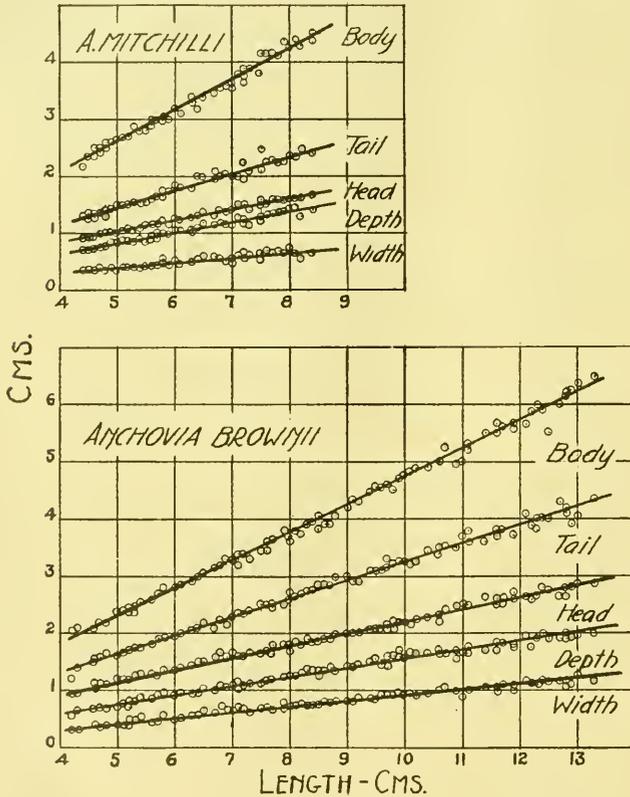
Figure 7

'tangents' (c) can be substituted for two of the length factors in the weight-length equation (p. 382). This equation then becomes

$$\text{weight} = K \times \text{length} \times \text{width} \times \text{depth}.$$

The values of the constant K are determined directly by a comparison of figures 1 to 5 with figures 6 to 10 or indirectly from

tables 2 and 3 by means of the following obvious formula express-
ing the relation between K and the constants for weight-length
(a), depth (c_d), and width (c_w):—

$$K = \frac{a}{c_d\,c_w}.$$

The values given in table 4 were calculated by means of this
formula, except that for Cynoscion regalis, which was deter-
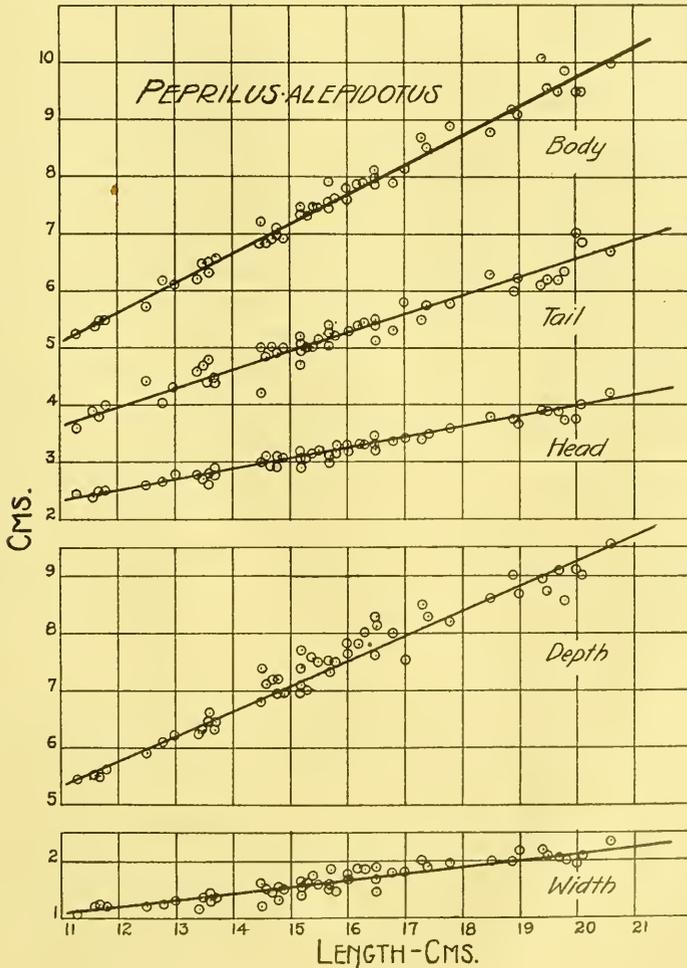
Figure 8

mined directly from the curves, and is taken from the paper
already referred to.

TABLE 4

Values of K

Brevoortia tyrannus.. 0.336
Anchovia brownii.. 0.549
Anchovia mitchilli... 0.475
Peprilus alepidotus.. 0.350
Cynoscion regalis.. 0.565
Leiostomus xanthurus.. 0.468
Orthopristis chrysopterus.. 0.467

RATE OF GROWTH

In the paper on the weakfish it was stated that the 'tangents'
of the lines, such as those in figures 6 to 10 (collected in table 3),
indicate the rates of growth of the various parts *relative to the
total length*. This statement as modified by the italicized phrase,
is correct, because the values for the constant *c* indicate the entire
number of units acquired by a given part per unit acquisition
by the total length.

Strictly speaking, however, these values do not represent
rates of growth, and the above suggestion is likely to be misin-
terpreted to mean that the real rates of growth of the various
parts are different. The rate at which a body or an organ
grows, is the number of units acquired in a given unit of time,
divided by the number of units already there (Minot, p. 93).
Applying this definition to the matter under consideration, it
becomes evident that the rates of growth of all parts of the
fish are identical.

This may be shown graphically by means of figure 11. In
A the total length of a hypothetical fish is represented by 20
units, the head being represented by 4 units, the body by 10
units, and the tail by 6 units.[4] The ratio of head to length is

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[4] These units have no morphologic significance, i.e., they do not represent
scales, because the number of scales is constant for any individual; nor are
they to be interpreted as representing cells. Compare in this connection the
work of Berezowski ('10) on the size of cells in a growing organism, Morgulis ('11)
on the size of cells during decrease of body size through inanition, of Morgan
('95 and '96) and of Driesch ('00) on the size and number of cells in embryos
from isolated blastomeres, and of Conklin ('12) on cell size and body size.

0.20, of body to length is 0.50, and of tail to length is 0.30.   In a
given unit of time the fish increases to the size represented by
B.   Since the form of the fish is constant, the relationships
between the total length and the length of head, body, and tail
are the same as they were in *A*.   It will be seen that the total
length has increased 10 units, the head 2 units, the body 5
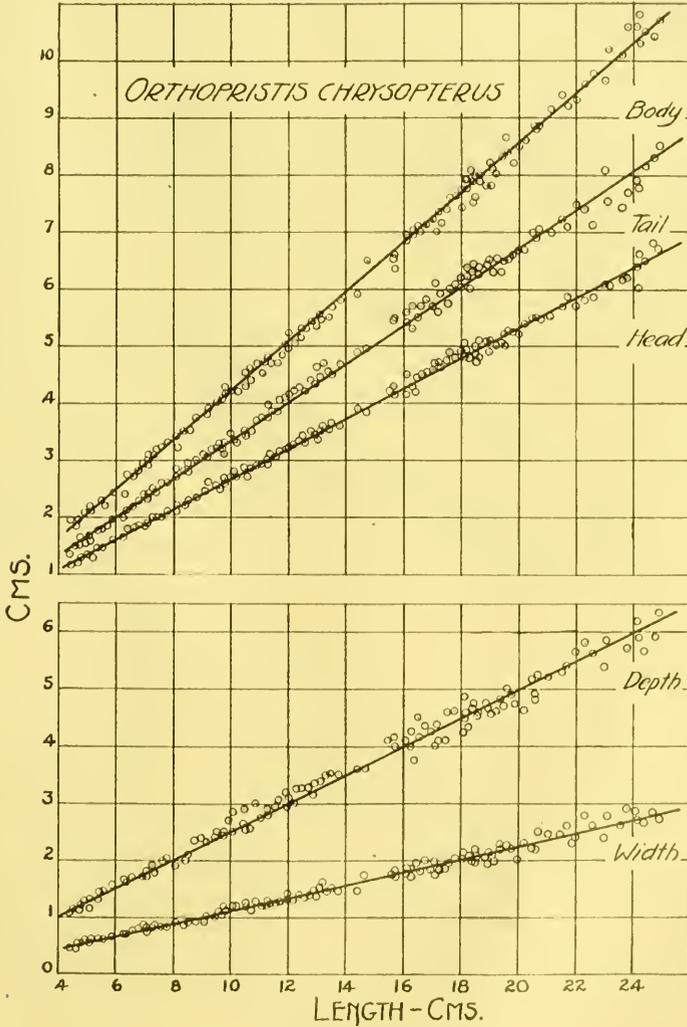
Figure 9

units, and the tail 3 units. *Per unit increase in total length,* the
head has increased 0.20 units, the body 0.50 units, and the tail
0.30 units.   If, however, the increase of each part be divided by
the number of units present in that part before the growth took
place, we find that there has been an increase of 50 per cent
in each part.   Hence the percentage increase in that unit of time
—in other words, the rate of growth—is the same for all parts
of the fish.
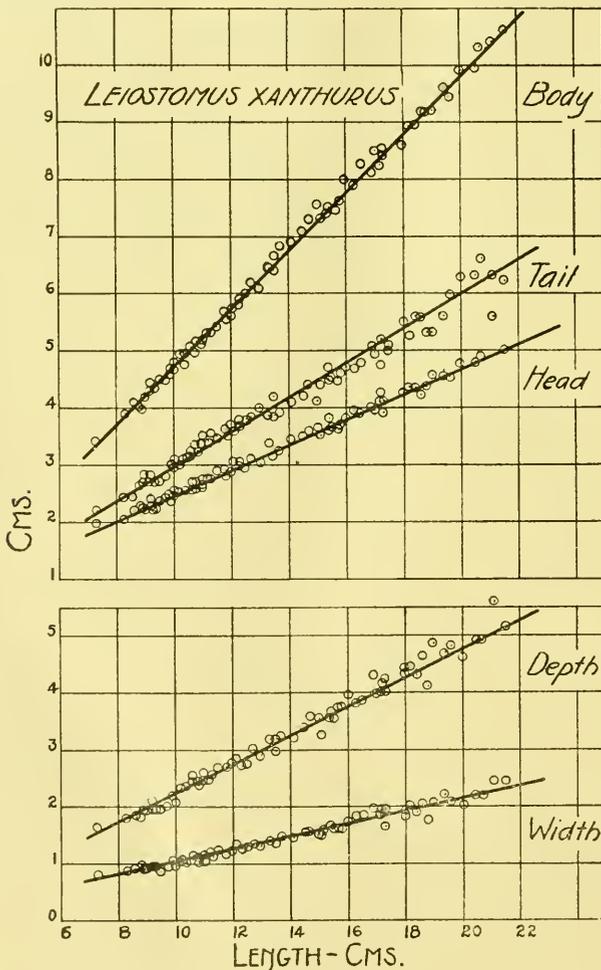
Figure 10

Donaldson ('98) and Donaldson and Schoemaker ('00), working with frogs, measured the lengths of the tibia, femur, and foot, and compared them with the total length of the leg-bones, and with the length of the frog. The results bear out the conclusion that the ratios of the lengths of the different bones to the total bone length, as well as to the total length of the frog, are constant for each bone, for all sizes of frog. These results are strictly in accordance with those given in the present paper.
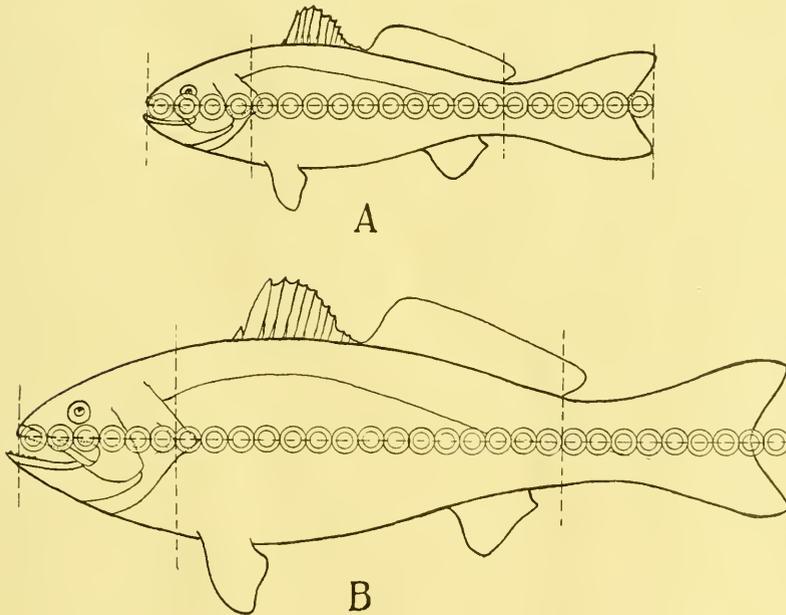
Figure 11

For the frog, then, the various leg bones, the total length of the bones, and the total length of the individual all have the same rate of growth. The curves given by Crozier ('14) for the interrelation of various parts of the shell of Dosinia are open to a similar interpretation, namely, that the rates of growth of the different parts of the shell are identical.

This conclusion is all the more interesting because it does not apply to the growth of higher vertebrates. The literature on the measurement of the growth of the surface parts of higher

vertebrates is very meagre, most of the work on growth having been concerned with the weights of the body and internal organs.[5] Sufficient data, however, exist to show that the rates of growth of the parts of the body of higher vertebrates are different for the different parts.

Topinard ('85, p. 1030) gives the measurements of the length of the head, trunk, thigh, leg, arm, and hand, in man from birth to twenty years. I have calculated from the values given, the rates of growth of these parts, and find that the parts have rates of growth differing widely from one another. Thus during the same period of time the head grows 55.8 per cent of its former length, the trunk 59.8 per cent, the thigh 78.7 per cent, the leg 69.5 per cent, the arm 60.6 per cent and the hand 52.3 per cent. Less complete measurements made by Menard ('85) on the horse indicate the same lack of conformity in rate of growth of the different parts.

In man not only are there differences between the parts of the body, but these differences do not remain constant. For, at different periods in the growth of the individual the value of the ratio between any given part and the total length varies considerably. This condition is in strong contrast to the constant and uniform relation found for fish, for the frog, and for Dosinia.

Thus it seems possible to group on the one hand fishes and probably frogs and Dosinia, in which growth is uniform but unlimited (indeterminate), and on the other hand man and other higher vertebrates, in which growth varies, finally ceasing altogether (determinate). In animals with indeterminate growth the form of the individual is laid down very early in life, and is adhered to, within narrow limits, throughout its period of growth —which means for the rest of its life. In higher vertebrates, however, the form is continually changing during the period of growth, and as soon as it becomes constant (the adult stage) growth ceases.[6]

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[5] For a summary and critical discussion of such growth curves, see Meyer. For a theoretical interpretation of them, see Hatai ('11).

[6] The nice point as to whether growth ceases because the form becomes constant, or whether the form becomes constant because growth ceases, I shall not attempt to discuss.

It must be added, however, that this conclusion applies only to external form, because Kellicott has shown that in the case of the dogfish, certain internal organs (brain and viscera) differ in their rates of growth in much the same independent way as in higher vertebrates.

FORM

A study of table 3 (p. 389) reveals some interesting points. It will be noticed that the values of the constants c for the length of the body, head, and tail, and for width (but not for depth) are surprisingly alike for the different species. For example, the values for the body-constant vary in the different species between 0.424 and 0.530, which is a fair agreement considering the different relations of the body limits in the different species. The values for the tail-constant show a still better agreement, the variations here being between the extremes of 0.273 and 0.334. In fact, if an average is taken for each constant, the extreme variation on either side of this average is approximately 10 per cent, while most of the values lie well within this range.

This general similarity of form in the different species is, of course, not unexpected. On account of the relatively dense medium in which fishes live, and the resistance it offers to movement, it seems reasonable to expect that, in general, all fish will present a form that offers the least resistance, and yet have the necessary mechanism for rapid motion, preying, defense, and so on. Individual variations in the degree of development of the various functions are bound to result in modifications of this generalized fish-form, and an expression of this variation is given in the figures of table 3.

A corroboration of my conclusion as to this general similarity of form is found in the work of Parsons ('88), who has studied the contours and areas of about fourteen teleosts and several cetaceans from the point of view of the naval engineer. He has found that if the areas of the cross sections of a teleost are plotted against their distances from the tip of the snout, the resulting area curves are surprisingly similar for all species. He states that "the position of the greatest area of cross-section is fixed

for all species, being situated at 36 per cent of the length behind the snout."

In striking contrast to the similarities shown by the other ratios, the values for the depth-constant vary considerably. A comparison of these with the weight-length constant, given in table 2, shows that, in general, a species having a high value for the depth constant has also a high value for the weight-length constant (e.g., Peprilus alepidotus) and vice versa (e.g., Anchovia brownii). It would therefore seem that the 'special' form of a fish, as contrasted with the generalized fish-form, is largely determined by its depth measurement.

SUMMARY

1. From the study of eleven species of fish, representing seven families, it has been shown that, in all of them, weight and length are closely correlated. This relation is such that weight is equal to a constant multiplied by the cube of the length:—

$$\text{weight} = a \times (\text{length})^3.$$

2. The factor a serves as a convenient index of the 'condition' of the fish at different seasons of the year.

3. Sex has no influence on these correlations.

4. In all the species, measurements of the selected external body parts are simple linear functions of length, and are constant.

5. A relationship is shown to exist among weight, length, width, and depth, such that the value of weight may be expressed in terms of the other three. The equation is

$$\text{weight} = K \times \text{length} \times \text{depth} \times \text{width},$$

in which K varies but little from species to species, and represents in each a modified value of the specific gravity.

6. The rates of growth of the different selected parts of the fish are identical.

7. Comparison with other data indicates that in animals having an indeterminate growth, the external form is established early in the post-embryonic life of the individual, and is adhered to, within rather narrow limits, for the rest of its life; whereas

in animals having determinate growth, the external from changes continually during the period of growth, and as soon as the form becomes constant, growth ceases.

8. The ratios giving the dimensions of the parts of the body in terms of length, show that the forms of the different parts (except depth) are quite constant in the different species, indicating their conformity to a generalized fish-form. This is correlated with the known similarity in the 'entering angle' (Parsons) of the different species.

9. Depth, however, varies from species to species, and seems to be largely responsible for the special form of a species.

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THE STRUCTURE AND GROWTH OF THE PLESIOSAURIAN PROPODIAL

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SIX TEXT FIGURES AND ONE PLATE

Plesiosaurian propodials[1] presenting unusual characters in the presence of grooves, foramina, cavities and canals have been known to paleontologists for some years and they have been commented on by Williston ('03), Lydekker ('89), Kaprijinoff ('82), Smith Woodward ('98) and the writer ('08).

Since few attempts have yet been made to explain the curious conditions found in the limb bones of plesiosaurs the writer wishes to take this opportunity of offering his views as to the meaning of these structures. The explanation offered below was developed in connection with the study of an immature skeleton of a new plesiosaur described some three years ago (Williston and Moodie '13) in a preliminary way.

That there may be no misunderstanding concerning the use of the word immature, it will be necessary to state that the plesiosaur exhibiting the conditions referred to above simply had not attained its full growth, as is evidenced by the immature conditions of the limb bones, in which the articular

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[1] The terms propodium, mesopodium and metapodium (which are equivalent to propodial, mesopodial and metapodial) were first used to indicate the separate portions of the molluscan foot as it occurs in the gastropods and pteropods, where it is divided into three distinct parts. The terms are equivalent to the propterygium, mesopterygium and metapterygium of the fishes. Since in the plesiosaurs there is very little structural difference between the skeletal elements of the fore and hind limb, it being impossible to locate isolated bones except by comparison, and since the bones of these animals are so commonly found disassociated, paleontologists felt the need of some term to indicate the limb elements without referring to its location on the body, so the terms propodial, mesopodial and metapodial were adopted. Propodial is the more common term, and indicates a limb bone which is either a femur or a humerus.

surfaces have not developed distinct facettes; the mesopodial elements are rounded and incomplete as though imbedded in a mass of cartilage, as well as the presence of growth characters which, heretofore, have been found only in young or embryonic bones. The species of plesiosaur referred to has been described as Ogmodirus martinii and the animal may have been twelve, to fifteen feet in length, which, in comparison with other members of its family (the Elasmosauridae) is not long. The Plesiosauria included some of the largest aquatic reptiles which have ever existed, equaled perhaps, though not exceeded by some of the extinct crocodiles. The largest known are probably those of the Kansas chalk, or the Jurassic of Wyoming, which probably reached a length of nearly or quite fifty feet, of which the neck formed about one-half (Williston, '14).

The elements of this incomplete skeleton which concern us here are the humerus, the femur and the phalanges. These are the only elongate bones of the limbs, the other elements being rounded plates (text fig. 1). In one of these, the femur, the bone has been broken across the plane of the medullary cavity, exhibiting the internal structure of the bone at this place (plate 1, fig. 3 and text fig. 4). The foramen, which is clearly evident from a lateral view of the bone, leads into an elongate canal, which, in turn, opens into an enlarged medullary cavity, which is filled with calcite in the specimen, and from this cavity the bone structure (plate 1, fig. 3) radiates in all directions. The outlines of the medullary cavity are not regular, but present many sharp pointed indentations which have been interpreted as being the places of exit of the Canales ossificantes perforantes.

The ends of the limb bones are covered with curious pits and cones which resemble miniature volcanoes 'A' (plate 1, figs. 2 and 4). The significance of these curious structures is suggested below. They are confined to immature bones. It has been generally assumed that all of these unusual characters mentioned above have been confined to the propodium, but, in studying the osteology of Ogmodirus it was noted that the phalangeal bones exhibited the foramina, canals and cavities,

with the same curious development of the articular ends as
in the propodium.   From what we know in the development of
mammalian long bones it would be expected that all long

Fig. 1.   Femur (propodial) of Ogmodirus martinii, Williston and Moodie,
from the Cretaceous of Kansas. $\times$ .5

Fig. 2   A longisection of the distal portion of an embryonic plesiosaurian
propodial, showing the sharply marked cone at A indicating a distinct differ-
ence in the rate of growth in the peripheral, or perichondral, and the secondary
or endochondral bone.   The cone-shaped structure A has been called an epiphysis.

bones in the extinct reptiles should exhibit these characters.
Further study of this problem will doubtless result in the
discovery of these characteristics in all of the plesiosaurian
long bones, especially in young and immature animals.

The writer believes that an adequate explanation of these interesting conditions is to be found in the developmental history of mammalian long bones.

The embryonic characters in developing long bones of mammals are so well known that little need be said here concerning them. Szymonowicz ('02) has figured in a developing long bone of a mammal an opening which he terms 'periosteal bud,' similar in all respects to the opening in the edge of the plesiosaurian propodial. In both cases a canal leads from the foramen into the medullary cavity. A similar observation is recorded in the text-book of histology by Böhm, Davidoff and Huber (p. 117, fig. 85) for a lizard embryo, and showing a much earlier stage in the development of the canal. These observations are readily confirmed on embryonic material; the pig showing the characters in a well-developed manner.

That this canal and opening persist for some time in foetal life is indicated by Jackson ('04) where he has figured and described a similar but more advanced condition in the tibia of a three day cat. Through the opening in the edge of the bone pass the bloodvessels supplying the medullary cavity, as well as the osteoblasts and the marrow-forming elements. It is very interesting to observe the persistence of this canal and foramen in mammals for that is exactly the condition in the immature plesiosaur referred to above (Ogmodirus martinii) as well as in all embryonic propodials of the plesiosaurs; with the important difference that this embryonic feature was retained much longer in the ancient plesiosaurs than it is in the modern mammals. There is no reason for doubting that the same processes of bone formation went on in Cretaceous times as are going on now, so the comparison is a safe one.

In all, or nearly all, young and embryonic bones of plesiosaurs, which are fairly common in the Cretaceous deposits of Kansas, there occur the above mentioned foramina, canals, cavities, and a well-marked groove, extending, in some cases entirely around the bone, and often persisting until late in life, being known to occur in an adult limb bone of Polycotylus. A representative embryonic propodial is figured herewith (text

fig. 5), the characters of which have already been fully described (Williston '03 and Moodie '11). In this specimen all of the growth characters above referred to, i.e., canal, foramen, cavity and groove, are present. The groove forms, save for a slight interruption, a shallow smooth-bottomed trough en-
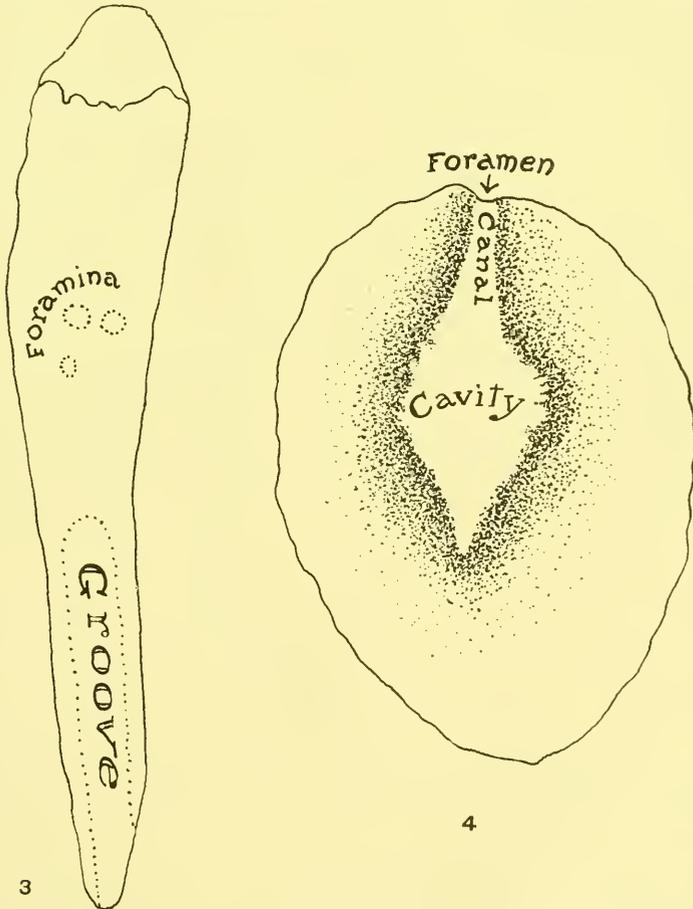
Fig. 3 A drawing of the edge of a plesiosaurian (Polycotylus) propodial showing the growth characters in the shape of a groove at the distal end and three foramina which later may have either developed into Volkmann's canals or have disappeared.

Fig. 4 Drawing of the cross-section of the femur (fig. 1) as it is shown in a broken condition in plate 1, figure 3. The foramen is the same as one of those shown in figure 3.

tirely around the periphery of the bone. On one side of the bone there are three vascular openings of some size as well as many smaller ones. In adult bones all of the characters disappear, the groove gradually being forced toward the distal end, where it may persist for some time, the cavity, canal and foramen being gradually obliterated.
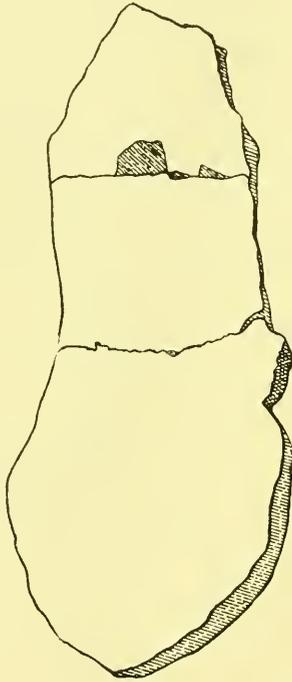
Fig. 5 An embryonic plesiosaurian propodial, from the Cretaceous of Kansas. This bone shows all the growth characters discussed in the present paper. × 1.

The groove on the edge of the bone can only be explained on the assumption that there are two ossific centers for the propodium, and that between the extension of these the cartilaginous endochondrium reaches the edges of the flattened bones. In the bottom of this groove a capillary bud has pierced the perichondral (periosteal) lamella and has invaded the endochondrium, thus forming the 'foramen.' Later piercing of this vessel (periosteal bud) results in the formation of the 'canal'

and the final invasion and spreading out of the arteries results in the formation of the medullary cavity. From this medullary cavity canals lead out to the extremities of the bone, as will be immediately described.

On the ends of the propodials of the immature Ogmodirus martinii, are well-developed rugosities which take the form of miniature volcanoes. (Plate 1, figs. 2 and 4). They are distributed over the entire articular surface of the bone. These openings and mounds are, doubtless, explained on the basis of bone growth in mammals. Bidder ('06) has offered an interesting explanation for the formation of epiphyses in mammals, by the migrations of the osteoblasts through special vascular canals (Canalis vasculosis perforans) which traverse the space between the medullary cavity and the cartilaginous caps at the ends of the limb bones. This condition is indicated diagrammatically for the plesiosaurs (text fig. 6), which is based on a study of broken and sectioned plesiosaurian limb bones. The periphery of the medullary cavity in the femur of Ogmodirus is very irregular and leading out from the sharp-pointed embayments are the Canales ossificantes perforantes, as is indicated in text figure 6. The blood vessels, and with them the migrating young mesenchymal cells—the osteoblasts—may have traversed these canals through the previously formed bone to enter the cap of articular cartilage. The action of the bone-forming elements was not, in this case, to produce new osseous growths (epiphyses) but simply resulted in the extension in the length of the bone. The mounds on the ends of the propodials look as if bony matter had been poured out of the small opening to harden around the orifice, exactly as lava does around the opening of a volcano. What really happened was that the osteoblasts, if they form bone, arranged themselves on the borders of the openings of the Canales ossificantes perforantes and there formed the bone which resulted in the extension in length.

The limb bones of the plesiosaurs, ichthyosaurs, mosasaurs, and some of the larger dinosaurs are solid, as they are in the modern manatees. This condition is, of course, due to the absence of an osteolytic element (presumably the osteoclasts) or the pres-

ence of these cells in small and ever diminishing numbers. In
explanation of the absence of these cells, or the lack of evidence
of their activity we find that only the more sluggish Mesozoic
reptiles exhibit solid limb bones. The more active theropodous
dinosaurs, the teleosaurs, the crocodiles, turtles and birds have
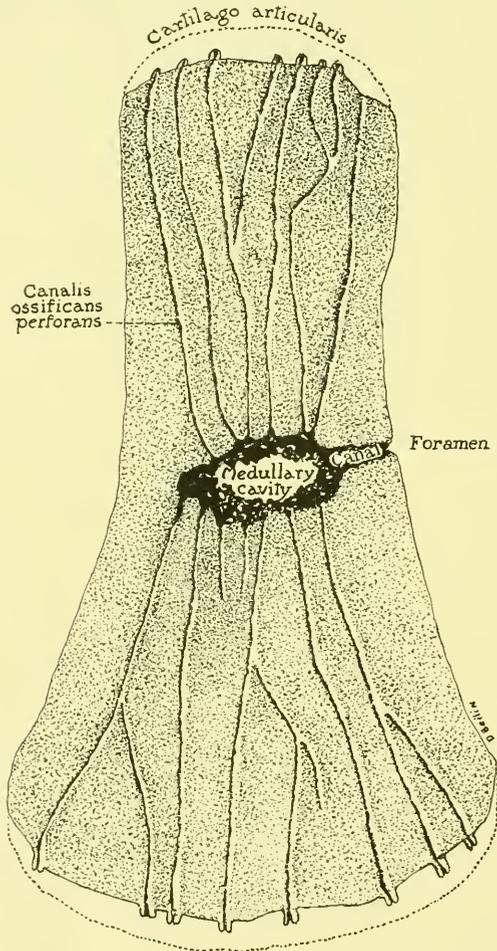
Fig. 6 A diagram based on actual material, showing in a medial section
the way in which the various growth characters are associated. The cones,
shown at the terminations of the 'Canales ossificantes perforantes' are shown
by photograph in plate 1, figures 2 and 4.

hollow limb bones. Why activity should result in the production of osteoclastic or other osteolytic elements is uncertain, but the evidence that such was the case is clear.

From the above discussion it is clear that the growth of bone followed precisely the same lines in ancient Mesozoic times as prevail today. The bone was doubtless preformed in cartilage, though no definite evidence of this is yet available. The osteoblasts arranged themselves first around the periphery and formed the perichondral (primary periosteal) lamella of bone, which was later pierced by a 'periosteal bud,' either at the time of formation of the bone or later by a process of erosion. The osteoblasts doubtless migrated, in the plesiosaurs, as in modern mammals through the opening made by the periosteal bud into the cavity formed in the endochondrium and there formed the secondary bone; which later became pierced by the Canales ossificantes perforantes extending to the ends of the bones. The cavity, canal and foramen later became filled up with bone, owing to the absence of osteolytic elements and resulting in the formation of a solid limb bone. Whether the foramen and canal resulted in the formation of a Volkmann's canal, is uncertain, but, since there are often evidences of more than a single canal, it is quite probable that one or more of these perforating canals through the primary lamella may have formed such a structure.

The growth of primary periosteal (perichondral) and the secondary (endochondral) bone in the plesiosaurs was quite distinct as is evidenced by specimens of embryonic and young bones, in which it is clear that the formation of bone has been produced by growth in different directions, resulting in a sharp cleavage between the layers of bone.

SUMMARY

Growth characters among the Cretaceous plesiosaurs followed, so far as the limb bones are concerned, essentially the same lines as do mammalian limb bones at the present time. The characters present in immature plesiosaurian propodials can be explained on the basis of embryonic characters of mammals. Solid limb bones, evidently only present in the more sluggish

and inactive types of reptiles, are probably due to the absence of an osteolytic agent, which, in other animals, produces the medullary cavity of adults. The primary medullary cavity of the plesiosaurs, due to the inroad of the branches of the 'periosteal bud' becomes obliterated, as do all other growth characters, i.e., canal, foramen, groove, and rugosities, very late in life, thus furnishing an interesting instance of the persistence, in the Mesozoic reptiles, of characters which at present are found to occur in modern mammalian embryos.

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PLATE 1

EXPLANATION OF FIGURES

1 Propodial (humerus) of Ogmodirus martinii, Williston and Moodie, from the Cretaceous of Kansas × 0.5 'S' = tooth marks of some predaceous fish or reptile of the Cretaceous seas.

2 Distal articular end of the humerus of the Ogmodirus showing 'A' the details of the small 'volcanoes' the ends of the 'Canales ossificantes perforantes.' × 0.75.

3 Cross-section of the femur (fig. 1) showing the foramen, canal and calcite-filled cavity. The bone structure radiates from the calcite-filled medullary cavity. × 1.

4 Proximal end of the humerus of Ogmodirus, showing growth characters 'A' in the form of small volcano-like eruptions. × 0.75.
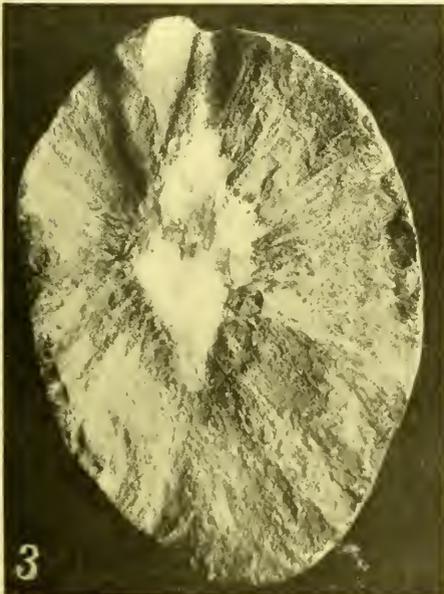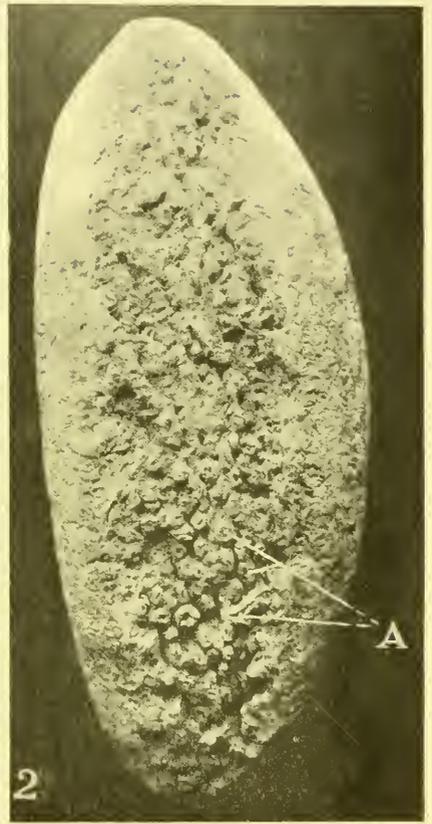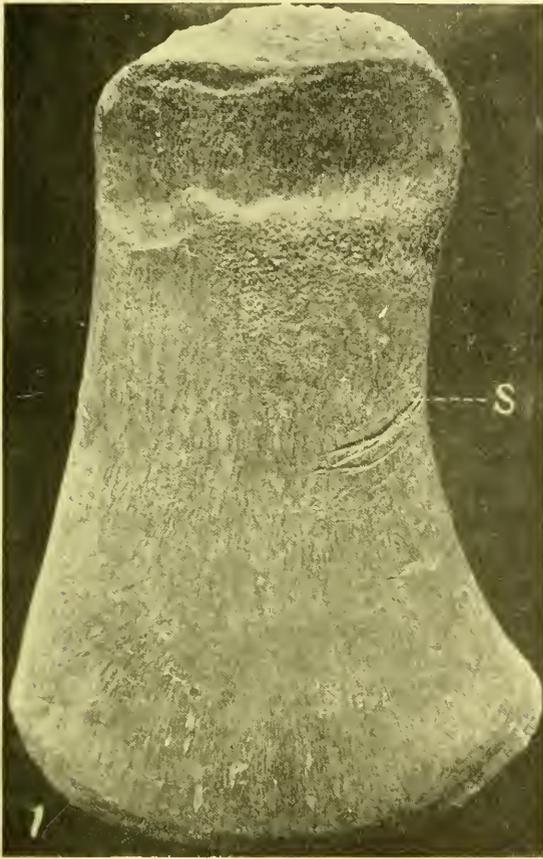
OPHIURA BREVISPINA[1]

II. AN EMBRYOLOGICAL CONTRIBUTION AND A STUDY OF THE EFFECT OF YOLK SUBSTANCE UPON DEVELOPMENT AND DEVELOPMENTAL PROCESSES

CASWELL GRAVE

From the Zoological Laboratory of the Johns Hopkins University, Baltimore, Maryland

FOUR TEXT FIGURES AND THREE PLATES

CONTENTS

INTRODUCTION

In a paper entitled 'Ophiura brevispina' which appeared in 1900 I published descriptions of the larva peculiar to this species of brittle-star in various stages of its development, but, for reasons stated in the paper, material was not then available from which to describe an unbroken series of the stages in the development of the larva, and the quantity of material representing some of the stages described and figured was not sufficient upon which to base positive conclusions with reference to the origin and fate of certain parts of the coelom.

[1] Ophioderma apparently has prior claim as the name of the genus and in recent lists, viz., in the "Biological Survey of the Waters of Woods Hole and Vicinity" by F. B. Sumner and others, Bull. Bureau of Fisheries, 1913, the name of the species is given as Ophioderma brevispina (Say.)

During the summers of 1900, 1901, 1902, and 1904, while at the Fisheries Laboratory at Beaufort, North Carolina, I was able to follow the development of living eggs and larvae of this species and to preserve an abundance of material from which it is now possible to complete the account of its development and to confirm or correct conclusions which, as first published, rested upon insufficient observation.

I am now able also to show that the organization of the egg of Ophiura, although seemingly different from that of other echinoderms, is in reality fundamentally the same. Since the apparent peculiarities in the organization and development of the egg of Ophiura are possibly to be correlated with the unusually large amount of yolk with which it is filled, a fairly complete account of the distribution, arrangement and form of the yolk within both the unsegmented egg and the cells of the early larva, and of the effect upon development and developmental processes traceable directly or indirectly to the yolk, is included. Certain observations on the breeding activities of the species which supplement those of the former paper are appended.

<div style="text-align:center">THE EGG</div>

The mature egg of Ophiura brevispina has a diameter of approximately 0.3 mm. and is therefore about seventy-eight times the volume of the egg of Ophiocoma echinata, the latter being not far from the minimum size of eggs produced by ophiurid species in general (text figs. 3, D and C.) The great difference in the size of the eggs of these two species seems to be chiefly, if not exclusively, due to a difference in yolk content and not to a difference in volume of ground substance.

The yolk material of the egg of Ophiura, like the yolk material of all echinoderm eggs, is distributed uniformly throughout the cytoplasm in the form of minute spherules and does not become stratified or localized within the egg either before or after maturation. The yolk spherules are practically uniform in size and, in the same brood of eggs, in color also, and they give to the eggs their characteristic tint. There is a striking varia-

tion, however, in the color of the eggs constituting broods produced by different females. The extremes in color noted in the broods of Ophiura eggs are lemon yellow and dark green.

EARLY DEVELOPMENT

The egg of Ophiura begins to segment in about one hour after it has been fertilized (extruded into the water). Its cleavage is total and, for a time, equal (text fig. 1, A–E.) In these activities and in the time relations also between the early cleavages
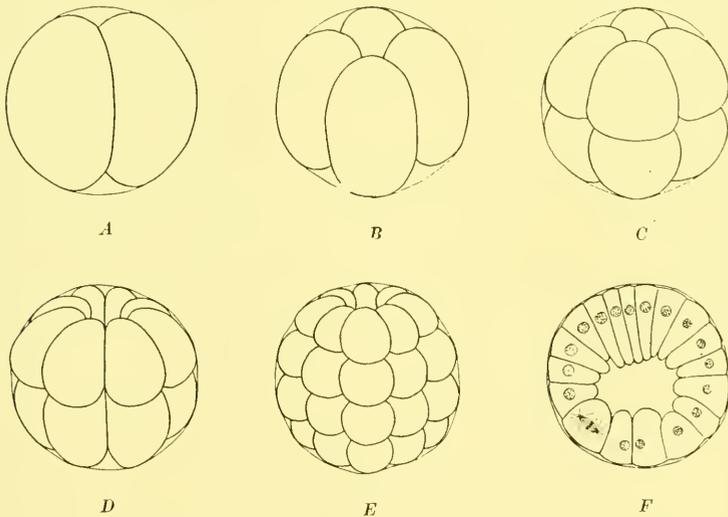
Text fig. 1 Outline drawings of early segmentation stages of the egg of Ophiura brevispina, A–E, and an optical section of the early blastula, F.

there is almost perfect similarity to the same phenomena observed in the development of the eggs of species of Ophiocoma, Ophiophilus and other genera which produce eggs of minute size. This close similarity in the early developmental processes of eggs of these two extreme types seems to indicate that cleavage in Ophiura has been disturbed but little if any by the great increase which has taken place in the yolk supply and size of the egg. That this yolk material is, or at least is correlated

with, a factor that *does* greatly disturb some of the developmental processes of the egg of Ophiura will appear later.

A segmentation cavity makes its appearance very early in the segmenting eggs and, during stages in late segmentation and early blastula, is somewhat eccentric in position on account of the more rapid multiplication and growth of cells situated at the animal pole than of those at the vegetative portion of the egg (text fig. 1, F).

BLASTULA AND MESENCHYME FORMATION

The blastula of Ophiura, in the stage during which the mesenchyme cells are being transferred from a position in the epithelial wall to one in the segmentation cavity, merits careful description because of the clearness with which its constituent cells show, by their form and structure and by their interrelations, the effect of a force, or forces, which takes part in this change in position of the mesenchyme cells.

By reference to figure 1, which is a camera drawing of a median sagittal section of a blastula fixed in a sublimate-acetic solution, it will be noted that the mesenchyme cells seem to be pushed or squeezed from the wall of the blastula into the segmentation cavity by a force exerted upon them from behind and from the side, by the adjacent parts of the blastula. As a result of this pressure the nucleus of each of the 'inwandering' mesenchyme cells has taken the form of a cone having its apex pointed away from the segmentation cavity. The small mass of yolk-free cytoplasm by which each nucleus is surrounded shows the effect of this pressure, even more than does the nucleus, and in every case it has the form of an elongated strand of material trailing behind its nucleus with its long axis perpendicular to the lines of force of the lateral pressure.

The membrane, by which the blastula is enclosed, is thrown into wrinkles and irregularities in thickness over the area involved in mesenchyme formation and thus also registers the effect of coercive pressure and constriction.

This condition of the nuclei and cell contents of the differentiating mesenchyme cells is not that which they would assume were they the initiators and self sufficient performers of their change in position but is that which might be expected if the organism as a whole is the dominating agent in the process.

It is not contended that these mesenchyme cells are entirely passive agents in the processes which result in their transfer from the wall of the blastula to the segmentation cavity, they being parts of the organism, but that the part they may take in bringing about their transfer is so much less than that played by the organism as a whole that it is entirely masked in this case. There is no indication whatever that these mesenchyme cells are wandering or migrating autonomously into the segmentation cavity.

In eggs containing a comparatively small quantity of yolk material, the mechanical processes required to bring about the transfer of mesenchyme cells take place under much less complicated conditions than in an egg, such as that of Ophiura, in which the quantity of inert yolk material is relatively so large in comparison to the quantity of active living substance associated with it. In the latter case the transfer of the mesenchyme cells can not take place until a relatively large amount of inter-cellular adhesion and friction has been overcome, hence processes not evident in eggs of smaller size and different composition here become magnified and apparent.

Working mainly with blastulae developing from eggs small in size and poor in yolk content, the numerous investigators of the mechanics of mesenchyme formation and invaginate gastrulation have discovered within the differentiating cells, or properly assumed to be present, several local factors, some operating in one case, another series in other cases, among which may be enumerated: differential growth and cell division, liquefactions, absorption of liquids, intracellular migrations, changes in permeability, surface tension, etc., and no one has been able to find conclusive evidence of the participation of any other more general differentiating activity. The demonstrated exist-

ence of these local activities and effects has been accepted by
some investigators therefore as evidence that the developmental
processes in question are wholly autonomous and not brought
about or controlled by the organism as a whole

The existence of local changes within the cells of a differentiat-
ing area, facilitating or contributing the movements or changes
in the cells, is to be expected, even though controlling or directing
stimuli for the migration or invagination process might be defi-
nitely traced to some more general source.

In the mesenchyme cells of Ophiura there is also evidence
of the effect of some of the above mentioned local factors. A dif-
ferential absorption of liquid, probably from the segmentation
cavity, has evidently taken place *provided* the comparatively
larger size of the nuclei of these mesenchyme cells (when com-
pared with the nuclei of the adjacent cells of the blastula wall)
is accepted as proof of such absorption by the cells, as the work
of Glaser ('14a) indicates it may be. See figure 1. If these cells
absorb liquid then it is probably safe to assume that other chemi-
cal changes and surface effects within these differentiating cells
would naturally follow.

The change which has taken place in the disposition of the
cytoplasmic and nuclear materials within these mesenchyme
cells, however, is not such as can be satisfactorily explained
as due to an autonomous intracellular migration. On the other
hand it seems to register the effect of coercive pressure. The
wrinkled condition of the membrane beneath the mesenchyme
area seems to be an especially faithful witness of such a general
force and it is as evident in the living as in the preserved blastula.

Cell multiplication may in some cases play a role in supplying
the necessary tension for material transfers or movements
in development but in Ophiura, as in Sphaerechinus (Morgan
'95) and Amphioxus (Morgan and Hazen 1900), there is no
evidence although dividing cells are numerous that they are
so located as to be differentially efficient.

In the section shown in figure 1, eight cells are in mitotic
division and in the entire blastula, from which the figured sec-

tion is taken, there are eighty-nine dividing cells in the epithelial wall and ten dividing mesenchyme cells.

The configuration presented by the blastula of Ophiura therefore indicates that various forces are active in the differentiation of its mesenchyme cells, among which is one, apparently the dominating one, that is not intracellular in origin, but one which actively involves the organism as a whole. It may be identified with a process such as that referred to by Professor Whitman in his well known essay entitled "The Inadequacy of the Cell Theory," and is in harmony with the idea contained in the 'Tension Hypothesis' outlined by T. H. Morgan in his 'Regeneration,' page 275.

THE EPIGASTRIC COELOM AND THE ORGANIZATION OF ECHINODERM EGGS

A satisfactory account of the origin and differentiation of the epigastric coelom of the larva of Ophiura could not be given in my former paper for reasons stated therein. In my new embryological material, however, there are larval stages intermediate between those formerly designated B and C, the study of which enables me now to settle the question of the origin of this part of the coelom and also to suggest an explanation for other obscure points in the peculiar organization of the egg and the larva.

As formerly described and interpreted ('00, pages 88 and 90), the internal organization of the larva of Ophiura, in the stages in which various parts of the coelom are first morphologically differentiated, is briefly as follows:

A pair of small hollow pouches is constricted from the free end of the archenteron, one pouch on the left and one on the right, homologous with the anterior pair of enterocoeles developed in the dipleurula larva of ordinary Ophiurids. A large unpaired pouch is in the process of constriction from the middle, ventral and left portion of the archenteron, which was at first interpreted to represent both members of the posterior pair of en-

terocoeles so universally found in echinoderm larva, but, since
the subsequent history of this pouch in Ophiura showed that it
passes wholly and exclusively into the adult hypogastric coelom
and takes no part in the formation of the epigastric coelom, it
became apparent, either that the suggested homology of this
larval pouch with the posterior pair of pouches was wrong, or
that the material representing the right member of the pair of
enterocoeles has, in consequence of its fusion with the left, been
completely changed in both position and function, for the usual
history of the pair of posterior enterocoeles of echinoderm
larvae is that the left member becomes the hypogastric coelom
of the adult and the right member differentiates into the epigastric
coelom.   In larvae of a slightly later stage (stage C) the epigastric
coelom was found in its proper position over the right side of
the stomach portion of the alimentary tract and its form and
structure were such as to indicate that it had not been derived
from any part of the large ventral anlagen of the hypogastric
pouch.   The only suggestion as to the origin of the epigastric
coelom, that seemed possible from the structure of the larvae
of these two stages, was that the right anterior enterocoele,
plainly present in stage B and not evident in stage C, had
migrated to a new position on the right side of the stomach,
which, in stage B, was unoccupied by any coelomic structure
but which in stage C contained a small epigastric body cavity.
The large five lobed structure which in stage B was originat-
ing from the left anterior part of the large ventral hypogastric
pouch was evidently to be homologized with the hydrocoele.

Passing now to a consideration of the results of the study
of material secured since the publication of the first paper, it
is perfectly clear that the epigastric coelom of Ophiura is not
derived from a displaced and metamorphosed right anterior
enterocoele, but is differentiated, in an even more unexpected
and unprecedented way, by an invagination of that portion of
the body wall (ectoderm) of the larva which immediately over-
lies the right side of the stomach portion of the alimentary
tract.   The drawings of both the reconstruction and the sec-
tion of larvae which show this structure (figs. 4 and 5) were

made from a specimen in which this epigastric invagination still retains its communication with the exterior. This larva also has the right anterior enterocoele in its proper position at the side of the oesophagus. With this correction the developmental history of the larva is the same as that formerly described.

In Ophiura the coelomic structures are thus shown to be differentiated morphologically not at one point from a single aggregation of cells, as is the usual case in ophiurid development, but at three points from three isolated groups of cells, as follows: one at the free end of the archenteron (the anterior pair of enterocoeles), one in the midst of and on the ventral and left side of the archenteron (the left member of the posterior pair of enterocoeles and the hydrocoele), and one situated entirely outside of the endodermal tract and within the ectoderm (the right member of the posterior pair of enterocoeles).

These observed differences between Ophiura and other ophiurids in the places of origin of their evidently homologous coelomic structures, and certain anomalous similar phenomena presented by the developmental history of certain other echinoderms, (text fig. 2, A-F), seem at first sight to indicate differences of organization of a fundamental nature, which are opposed to the idea of the genetic continuity of the group of echinoderms, but, when viewed in the light of recent observations and experimental results, they not only cease to present difficulties in the way of unifying the group, but provide a basis for a further advance in our knowledge of the fundamental organization of the echinoderm egg.

It was first shown by Morgan ('94) and later more definitely by Boveri ('01) that the cytoplasm of the eggs of certain species of sea urchin is differentiated into or contains different kinds of material (formative stuffs), even before fertilization has taken place, and that these materials become localized after the maturation divisions into zones around the axis of the egg, one zone occupying a position at and around the vegetative pole and containing the material which is distributed during later development to the mesenchyme cells; a middle zone of material which is invaginated during gastrulation and distributed within

those cells which form the archenteron (mesoderm and endoderm), and a zone situated at and around the animal pole of the egg containing material for the ectoderm cells.

Morgan and Spooner ('09) have since clearly shown that these visible formative stuffs are not the determiners of the mor-
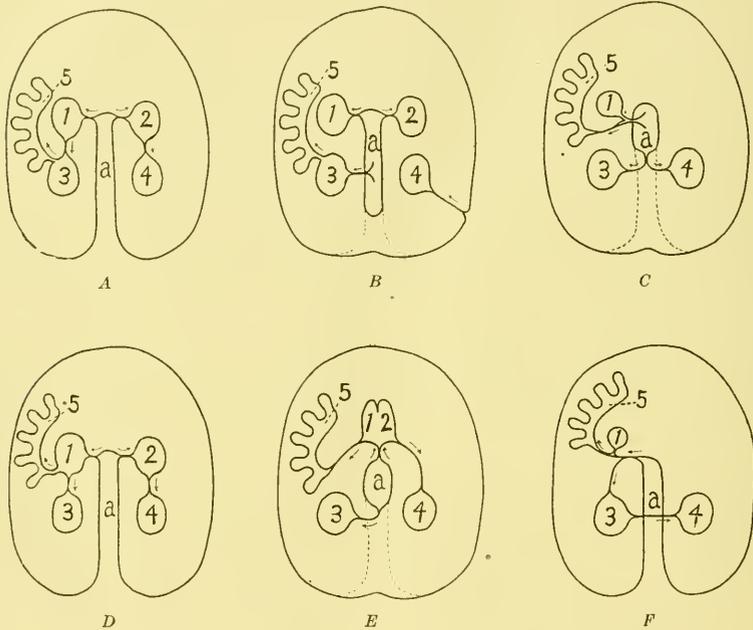
Text fig. 2 Schematic drawings to show the place and method of origin in development of certain parts of the coelom and the hydrocoele in the late gastrula stage of various echinoderms.

A and D are representative of the manner of differentiation of these mesodermal structures typical for then umerous species of Ophiurids, Echinids and Asterids which produce eggs of small size with comparatively small supply of yolk material.

The remaining figures represent what has been found to be the order of events in the development of certain species producing large yolky eggs: B, Ophiura brevispina; C, Antedon; E, Solaster; F, a holothurian.

In B, C, and E the blastopore closes soon after gastrulation, and the archenteron looses its connection with the wall of the gastrula. The dotted lines show the position formerly occupied by the archenteron and blastopore.

a, archenteron; 1, left anterior enterocoele; 2, right anterior enterocoele; 3, left posterior enterocoele; 4, right posterior enterocoele; 5, hydrocoele.

phological differentiations which follow upon development but are only the result of differentiations of the more fundamental underlying ground substance of the egg. The fact remains therefore that there lies back of the 'formative stuffs' of the unsegmented egg real differentiations of the ground substance of at least three kinds, and that they have, prior to morphological development, a localization about the axis of the egg the same as that shown by the visible formative stuffs.

The developmental histories of the numerous echinoderm larvae which have been studied indicate strongly that a promorphological differentiation of the ground substance and localization of formative materials, such as that shown to exist in Arbacia and Strongylocentrotus, holds true for and is characteristic of the eggs of echinoderms in general. Embryological studies indicate further that the middle zone of this predifferentiated material is composed of at least two distinct kinds of substance, the first being that which is destined to pass into mesodermal (coelomic) structures, the second to endodermal organs. In practically every case which has been followed, the coelom and its derivatives are differentiated out of material localized in a group of cells which, in the gastrula, occupies the distal or free end of the archenteron. These materials separate from the endodermal part of the archenteron in the form either of a bilateral pouch or a pair of pouches at a time just before the remaining proximal portion of the archenteron unites with the ventral ectoderm to form the mouth. In practically all echinoderm larvae this first enterocoelic pouch or pair of pouches contains all of the material out of which, by a definite series of constrictions or divisions, the entire series of mesodermal or coelomic structures of the adult are differentiated.

The great differences between Ophiura and other ophiurids in the places of origin of evidently homologous structures, and the anomalous organizations and peculiarities of the larvae of Solaster, Antedon and all echinoderms producing large yolky eggs (text fig. 2, C, E, F), may be satisfactorily harmonized and accounted for if we make certain fully warranted assumtions: first, that each of the coelomic structures, common to

all echinoderms, is represented by a specific part of the ground substance predifferentiated in a definite region of the egg; second, that these different substances, which ordinarily flow together after the maturation divisions to form the lower stratum of the middle zone of 'formative stuffs' and hence are carried in together during gastrulation, fail for some reason to become thus localized in the yolky eggs of Ophiura, Solaster, Cribrella, Antedon, etc., and hence are not all carried forward together during gastrulation; third, that the failure of these specific substances to be united into one continuous mass is due to an interference with the free action of the mechanical processes which normally bring about localization, by the inertia of the dense mass of yolk material with which the cytoplasm is filled; fourth, that the eggs begin to segment before the retarded localization processes have been completed and thus prevent their further action.

Localization, however, is not altogether prevented, for in Ophiura some of the mesodermal material (anterior pair of enterocoeles) becomes morphologically differentiated at the same place as in other echinoderms, but that part which forms the remaining coelomic anlagen lags behind, some of it occupying a place in the midst of the endodermal substance while some of it fails even to get into the middle zone, but remains in the midst of the upper polar zone of ectodermal substance.

By a comparative study of the development of the eggs of those species which, in the places of origin of the essential coelomic structures, differ from Arbacia or Strongylocentrotus, it should be possible to throw considerable light upon the fundamental organization of the echinoderm egg, for, by noting the various points at which each of the several mesodermal structures makes its first morphological appearance and the relation it bears to other larval structures, the approximate location within the undeveloped egg of the original material out of which each takes its origin can be established. The amount of material available for such a comparative study is large. More or less divergence from the normal sequence in the origin of mesodermal structures can be anticipated in the development

of the eggs of all of those echinoderms in which the supply of yolk material is large. Some idea of the character and value of material of this kind for such a study may be had from text figure 2, A-F.

A factor or condition of development of considerable importance, present to a greater or less degree in the early developmental stages of all animals, may be defined as the mechanical operation of changing the position and arrangement of the yolk material within each cell both before and after each cell division. It involves the expenditure of a relatively considerable amount of energy in processes which can not be regarded as essentially developmental in character.

The existence of a condition such as that referred to is made strikingly apparent in the egg of Ophiura during its early development because of the presence and uniform distribution throughout the cytoplasm of a relatively very large amount of yolk material which must be manipulated by the cells of the segmenting egg.

The relatively enormous amount of movement and rearrangement of the yolk spherules which takes place in the cells, both preceding and following every mitosis, may be inferred from figures 2 and 3, which are from camera sketches of portions of sections of a blastula fixed in an osmic acid solution; one cut transversely to the long axis of the cells and passing through the cells at the level of their nuclei; the other a section of cells in the plane of their long axes. Each drawing shows a number of cells in their resting condition and two cells in mitosis, one of the latter in a stage of division just at the close of anaphase. It may be noted that the cells not in mitosis have the form of slender prisms in which the ratio of the length of the long axis to that of the transverse is about as 9 is to 1. The nuclei are situated near the basal ends of the cells, are oval in outline and each almost fills the space between the side walls of the cell. The yolk spherules of each cell are separated by

the nucleus into two groups. That group which occupies the portion of the cell between the nucleus and the base of the cell, is composed of a relatively small number of spherules each surrounded by, or suspended in, a relatively larger amount of cytoplasm than is to be found between the spherules composing the larger group included within the portion of the cell situated internal to the nucleus.

The cells in mitosis are approximately spherical in form. The yolk spherules during this phase are arranged around the dividing nucleus in a continuous peripheral zone, which, on the side of the cell nearest the basement membrane, is one spherule in thickness and increases in thickness to three or four spherules on the opposite side.

The position of cells in mitosis within the epithelium of the blastula in all cases observed, is at the periphery. That part of the cell wall which is in contact with the basement membrane of the blastula, apparently forming a part of this limiting membrane, retains its original flat contour during the division of the cell, showing that this part of the cell is firmly attached or cemented to the basement membrane, or to the bases of the adjacent cells, and that the cells of the blastula are not free to move about or change their positions with reference to one another.

The relatively enormous change that takes place in the shape and position of a cell during mitosis greatly affects the form and condition also of the cells adjacent to it. The latter, in their adjustment to their spherical dividing neighbor cell, become bent, twisted and constricted and their nuclei become flattened or pushed from their usual positions, as shown by figure 2.

Yolk segregation. It would seem that the larva is in some way disturbed by the outgo of energy, and the hindrance to development, involved in the juggling of its yolk material, for, when we come to a later stage in the development of Ophiura, (the late gastrula in which the alimentary canal and coelom become differentiated) we find by an examination of the cells that make up its epithelia and form a part of the contents of the segmentation cavity, that a complete change in the dis-

tribution and form of the yolk material of the organism has been effected.

By reference to figures 5 and 6 it will be seen that by far the greater number of the yolk spherules orginally contained within the cells have been extruded and are now to be found lying free in the segmentation cavity. Each cell, however, has retained in its cytoplasm a small amount of its original supply of yolk. The yolk material that has been extruded from the cells is apparently the greater part of that which formerly made up the numerous group of spherules located in the parts of the cells situated between the nucleus and the segmentation cavity, for now this group in each cell consists of a comparatively few spherules. Apparently all of the yolk spherules which composed the group situated between the nucleus and the base of the cell have been retained within the cells.

These figures are reproductions of drawings of cross sections of a larva in the stage of development in which the coelom and its derivatives are first differentiated, such as is shown in figure 4. A further study of the sections shows that the yolk spherules, so long as they remain within the cells and are surrounded by cytoplasm, are relatively small particles and quite uniform in size, but that they fuse together to form much larger masses of varying size and shape when extruded into the segmentation cavity where they are practically free from cytoplasm.

Among the free yolk masses in the segmentation cavity mesenchyme cells may be seen here and there. Possibly the function of these cells is that of ingesting the segregated yolk material and redistributing it to parts of the larva where energy is most rapidly expended.

The result of the yolk segregation process in Ophiura is that a more or less complete separation of the active living substance of the developing individual from the inert non-living yolk material is effected, enabling the living substance thereafter to continue its development practically independent of and unhindered by the yolk; independent to this extent at least, that the individual cells are no longer under the necessity of expending energy in a constant manipulation of yolk.

Yolk segregation in other echinoderms. The eggs of practically all species of echinoderms are minute bodies and the small amount of yolk material they contain seems to interfere but slightly if at all with either the activities of the cells of the developing larvae or with other processes of development and differentiation. In all cases observed·the yolk material of echinoderm eggs is uniformly distributed throughout the cytoplasm and retains the same uniform distribution within all cells resulting from segmentation of the egg until it is finally absorbed during larval development.

Ophiura brevispina (and probably all species of the genus Ophiura) and a comparatively few species scattered here and there among various genera of all classes of echinoderms (Asterina, Cribrella, Solaster, Thyone, Antedon, etc.) differ from the more typical species of the phylum in that they produce eggs of relatively large size, containing a rich supply of yolk material.

The distribution and fate of the yolk material during the development of each of these species merits more careful study than it has yet received, and it is not a subject concerning which generalizations can safely be made. A comparison of the observations that have been recorded by Masterman ('02) on Cribrella and by Gemmill ('12) on Solaster with those of this paper, indicate that some sort of yolk segregation process may be expected to take place in the development of all yolky eggs of echinoderms but that each species has independently developed a segregation process peculiar to itself and without genetic relationship to that of other species.

Cribrella. In Cribrella, according to Masterman, both the segmentation and archenteric cavities of the larva become filled, soon after gastrulation, with cells which he designates mesenchyme and hypenchyme respectively. Yolk globules of varying size and shape are shown by Masterman (figures 26, 27 and 28) in all cells of the late gastrula; epiblast, hypoblast, mesenchyme and hypenchyme, but it does not appear from either figures or text that yolk material is present in relatively greater quantity in the mesenchyme or hypenchyme cells than in the

cells composing the epithelia. Whether the yolk of the seg-
mentation cavity continues to be enclosed within the mesen-
chyme cells or whether it may come to lie free in this cavity
can not be determined from the figures. All of the yolk material
shown in the larval cavities seems to be satisfactorily accounted
for as belonging, or as having belonged, to the invading cells,
and evidence is wanting to show that yolk segregation in this
species is effected in any other way than by a great increase in
the number of mesenchyme cells produced. There is no evi-
dence that the yolk laden cells of the larvae, as a whole, extrude
yolk into the blastocoele and archenteron as is the case in
Ophiura.

Solaster. According to Gemmill the yolky egg of Solaster
endeca has an equatorial diameter of 1 mm., a vertical diameter
of 0.8 mm. The gastrula has a large empty archenteron and
a small narrow segmentation cavity completely filled with
stellate mesenchyme cells. As development proceeds, however,
mesenchyme cells, consisting in chief part of large sized yolk
granules, continue to be budded into the coelomic spaces from
the basal aspect of the cells lining the anterior and posterior
coelomic pouches. All cells of the younger stages contain yolk
material, but, when stages of late metamorphosis are reached,
the cells forming the growing enteron of the larva contain at
least half of the remaining yolk, and from this fact it is inferred
that a gradual transference of yolk material from mesenchyme
cells to entoderm cells must have taken place.

Here again we have a species in which mesenchyme cells
have assumed the function of secondarily receiving yolk material
from other cells, thus segregating it for a time and finally ef-
fecting its transference to other cells.

Gemmill records observations on certain larvae, considered
by him to be possibly abnormal, which are of special interest
in connection with a consideration of the yolk segregation process
found in Ophiura. During the rearrangment of the cells of
certain blastulae of Solaster a few yolk granules were pressed out
of the cells and in the living specimens were seen eddying hither

and thither between the fertilization membrane and blastula. These granules were ultimately swept into the blastopore to form a plug.

Franklin Islands Asterid. From a single 'brood' of eggs and embryos, collected off the Franklin Islands, J. E. Henderson ('05) has described certain intermediate stages of the life history of an unnamed asterid, the structure of which indicates that the comparatively enormous quantity of yolk with which the egg is stored, either remains outside the blastomeres during early development, as in meroblastic cleavage, or is extruded from the cells later, as is the case in Ophiura. The following quotations from Henderson's paper show that a process of yolk segregation has been developed by this species, evidently very different from any hitherto found in an echinoderm. "The chief difference between the Asterina embryo and that of the Franklin Islands species is that the latter is much larger in size and have all the interstices of the body gorged with yolk." "The yolk forms by far the largest part of the whole star-fish, at least nine-tenths of the whole bulk is yolk. Stained with eosin this becomes easily differentiated from the other tissues. It is composed chiefly of large globular or irregularly shaped masses, which are closely apposed to one another, leaving few chinks intervening. This yolk not only fills up the center but penetrates everywhere; in fact, the whole of the tissues would seem to have been built up around it as, indeed, they really are. It is found among the ectoderm cells, between the ectodermic wall and the peritoneal wall of the coelomic spaces, and among the cells of the gut-wall, while with the gut it composes the main mass of the interior." "The gut-cells are oblong in shape with irregular ends towards the lumen, and seem to be engulfing yolk, as particles of yolk can be seen not only among them but also in their substance." "Amoebocytes and tissues of the mesenchyme" are found among the yolk in the segmentation cavity.

Yolk segregation in other phyla. If we turn to representatives of other phyla of the Animal Kingdom for examples of localization and segregation of yolk substance during or preceding

development, similar in effect to that observed in Ophiura, we find them in abundance among the Mollusca, Arthropoda and Vertebrata. In each of these phyla the same result is accomplished in a way different from that which has been developed in the others, but within each group the method of yolk segregation is essentially the same. In species of molluscs the yolk material is chiefly localized within one portion of the egg and becomes included during segmentation within a comparatively few inert cells. Among arthropods and vertebrates the living substance of the egg withdraws more or less completely from the yolk material and undergoes development and differentiation practically independent of it. In all of these cases the general effect of the segregation of the yolk substance is essentially the same; the energy of the developing organism is thereafter not expended in a constant manipulation of yolk, but is available wholly for activities that result in development and differentiation.

The processes of yolk segregation as they are found in numerous species of various genera of Gasteropods are so nearly identical in every way, including the particular cells involved, that it is difficult to conceive of them as not as much a part of the common inheritance of the group as are its common structural characters. The same may be said of the modes of yolk segregation peculiar to insects, reptiles and birds, but in the cases of Ophiura and other widely separated species of echinoderms the processes seem to have been developed so abruptly, are so isolated and different as to lead to the conclusion that they have been independently acquired and are not genetically connected. A specific process or mechanism of yolk segregation, needing only the stimuli of a changed internal environment (increase of yolk) to bring it into function, does not seem to be inherent in the echinoderm egg, because apparently the same internal environment which brings into function a process of segregation of one type in Ophiura, results in segregation of different types in the yolky eggs of other echinoderms.

Cerianthus, Actinia, Urticina. The Coelenterate phylum was not included in the foregoing list of those in which characteristic

processes of yolk distribution take place during or preceding development for the reason that the process as commonly found among coelenterates is somewhat obscured by other developmental phenomena and has therefore been subject to widely different interpretations. It is in this phylum, however, that cases of yolk segregation have been described which seem so nearly parallel to that which takes place in Ophiura as to merit careful comparison.

Kowalevsky ('73), Jourdan ('79–'80) and Appellöf ('00) have described and figured stages in the early development of Cerianthus membranaceus, Actinia equina and Urticina crassularia, respectively, in which the originally empty blastocoele or archenteron, or both, later become filled with free yolk material. Kowalevsky found in Cerianthus membranaceus that, after a typical invaginate gastrula has been formed from a one layered hollow blastula, the entoderm 'secretes' a mass of free yolk substance into the archenteron. Jourdan describes an invaginate gastrula also for Actinia equina and states that the free yolk which appears in both blastocoele and archenteron seems to 'exude' from the larval tissues, but Appellöf, after studying the development of this species, failed to find an invaginate gastrula, and, after critically examining Jourdan's figures, concluded that Jourdan was mistaken, in part at least, in the interpretation of his observations. Both investigators agree, however, that in this species a once hollow blastula later becomes filled with free yolk material. Appellöf has followed the process of yolk transfer as it occurs in Urticina in considerable detail, and as this is the only case which has been studied carefully, the following brief resumé of his observations is given: In Urticina crassicornis the unsegmented egg has a structure similar to that of typical coelenterates, a peripheral layer of clear cytoplasm and a central portion filled with yolk material. During segmentation a blastocoele appears and from the first this cavity contains a small quantity of granular material (central yolk substance) which is not incorporated within the blastomeres. The completed hollow blastula consists of a single layer of high columnar cells, each with its outer end made up of clear

cytoplasm and its inner portion crowded with yolk spherules. Then begins a process, which continues also during the invagination of the endoderm, of yolk segregation which is strikingly similar in certain respects to that found in Ophiura. The inner ends of the cells which contain the greater part of the yolk material become constricted into the segmentation cavity. The constricted ends of the cells contain no nuclei and are not split off from the cells by or during mitosis. They soon disintegrate into their component granules and spherules, thus forming a general mass of free yolk material. Then follows a process, as gastrulation proceeds, by which the yolk material of the blastocoele flows into the archenteron, and Appellöf believes that it passes between the entoderm cells as the latter gradually move toward the ectoderm, thus obliterating the segmentation cavity.

Appellöf's interpretation of the significance of this process of yolk segregation in Urticina is the same as that I have given to it in Ophiura, the large amount of yolk in the cells of the developing organism offers a very considerable hindrance to developmental processes in general and especially to those that involve the folding of epithelia, such as gastrulation, and, by separating the greater part of the yolk material from the cells, the larva is emancipated from this hindrance.

The differences in the processes as found in Urticina and Ophiura seem to be only those conceivably due to differences in egg organization and larval structure in the two species. In Ophiura the yolk material of the unsegmented egg is equally distributed throughout the cytoplasm, whereas in Urticina it is localized to a certain extent in the central part of the egg. In Ophiura the segmentation cavity persists throughout larval development and the greater part of the yolk material remains within this cavity until absorbed, a small portion only of the yolk being found in the archenteron.

Appellöf refrains from discussing the significance which the method of development found in Urticina may have in coelenterate development in general, but he suggests, not without seemingly good ground, that the planula larva, which is typical

for the phylum, is not to be regarded as a gastrula, but that it is the modified blastula of species producing eggs heavily charged with yolk material. Primitively the blastula of coelenterates may have developed into the gastrula stage by a typical process of invagination, but as the yolk content of the egg increased gastrulation became more and more hindered until, finally, it could only take place following a segregation of a portion of the yolk of the entoderm cells into the segmentation cavity (Urticina). When the yolk content of the egg still further increased, the entire space of the segmentation cavity was required to contain it, and, in response to such a condition, which is the common one among coelenterates, the planula larva was developed in which the entoderm cells take their definitive position not by invagination but by delamination or migration.

CORRELATION BETWEEN YOLK CONTENT OF EGG AND DURATION OF DEVELOPMENT

Various echinoderms differ greatly in the size of their eggs and also in the duration of their larval periods of development, and a considerable mass of embryological data seems to indicate that, in the absence of brooding of the offspring by the parent, a fairly definite correlation may exist between these two factors such that any increase or decrease in the first is accompanied by a corresponding inverse change in the second.

The existence of some sort of correlation between yolk content of egg and character of development throughout the animal kingdom is a matter of general observation and many references to it are to be found in papers on embryology. Students of embryology expect to find a modified development in all cases of yolky eggs and a developmental period of shortened duration in all cases in which such eggs are not in some way protected or cared for during development.

As stated above, the eggs of Ophiura brevispina (0.3 mm. in diameter) and Ophiocoma echinata (0.07 mm. in diameter) probably represent the extremes in a series of eggs of ophiurids (fig. 3 C and D), arranged as to size but, when the eggs of

echinoderms of all classes are considered, the egg of the star-fish Cribrella oculata (about 1 mm. in diameter) is probably as large as is known, and the egg of the sea-urchin, Toxopneustes variagatus, (about 0.065 mm. in diameter) probably stands near the other end of the echinoderm series.

Size of egg may depend upon more than one factor, one egg owing its superior size to a relatively greater amount of living or ground substance than another, but, with an exception to be noted later, the differences in the size of the eggs of echinoderms are due almost exclusively to differences in yolk content and not to differences in volume of ground substance.

Conklin ('07) finds an even greater diversity in the sizes of the eggs of gasteropods than is found among those of echinoderms, the egg of Fulgur being two thousand times the volume of the egg of Crepidula plana, and he has observed that the difference in the sizes of the eggs of gasteropods is also due almost exclusively to increased quantity of the yolk.

After a comparative study of the influence of increased volume of yolk substance upon development in gasteropods, Conklin concludes that it has produced little effect upon the organization of the egg or upon cleavage, but that it profoundly modifies the processes of gastrulation and organ formation.

In Fulgur and numerous other gasteropods, any effect the increased volume of yolk might have upon the rate and duration of larval development is neutralized by the protection which is afforded by the parent to the eggs and embryos during the entire developmental period, the young gasteropods escaping from the egg capsules in the adult condition. The cause for such a correlation is obscure, but that the correlation is real seems to be established by many observations.

The highly specialized larval forms of echinoderms (bipinnaria, auricularia, echinoplutei and ophioplutei), having comparatively long periods of larval existence, terminated by a more or less abrupt metamorphosis, develop from eggs in which the supply of yolk substance is relatively small. On the other hand rapidly developing larvae, simple in external form and without

an abrupt metamorphosis, are characteristic of those species which produce large yolky eggs.

Eggs of the yolky type contain an amount of reserve material and energy sufficient for all of the processes of development, hence larvae which come from such eggs develop rapidly into the adult condition, but, in the so-called alecithal type, the supply of available nutritive material in the egg becomes exhausted before development has proceeded far and larvae developing from such eggs are therefore under the necessity of spending a very considerable portion of their inherited energy in the acquisition of an additional supply of material with which to complete their development and for perfecting the larval mechanisms essential to their struggle for existence as independent organisms. The larval forms live in an environment totally different from that of the adult, hence structures and functions different from the corresponding ones of the adult are required by the larvae for capturing, digesting and assimilating food, for locomotion, for escaping enemies, etc.

It may be assumed that the time required for developing, perfecting and maintaining these extra-larval structures and functions is, in some way, added to the period required for the purely developmental differentiations and that the periods of larval development are thereby lengthened to degrees proportional to the complexity of the extra-larval structures and activities for which material and energy must be provided.

It is perhaps possible therefore roughly to indicate the correlation which exists between the yolk content of eggs and the periods of time required for their development, by a curve, such as that reproduced in text figure 4, in which the spaces along the axis of ordinates represents units of egg-diameter and those along the axis of abscissae represent unit periods of time.

This four-point curve, constructed on the basis of measurements and observations of the eggs and developmental histories of Ophiura (Grave), Ophiothrix (McBride), Mellita (Grave) and Toxopneustes (Tennent)[2] cannot be considered an accurate

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[2] Unpublished data kindly supplied by Dr. D. H. Tennent.

representation of the correlation phenomenon under consideration for several reasons, the most important of which are enumerated below, but it is the best that can be constructed from the data now available. The number of species for which both the size and character of mature egg and length of larval life are known is few. Certain species, of which these facts are known—Asterina (McBride), Cribrella (Masterman) and Solaster (Gemmill)—can not be placed in the scheme because in the first two the eggs and larvae are brooded and are hence freed from the larval struggle for existence, and, in the last, the adult form possesses nine instead of five arms and must therefore require much more energy and material for its development than is required in five rayed forms.

All species of star-fish are, for another reason, excluded from the correlation curve. With respect to volume of living or ground substance, the eggs of Asterids seem to belong to a series very different from that which includes the eggs of Echinoids and Ophiurids, the former containing a consistently greater volume of living substance. This fundamental difference be-between the eggs of the two series is evident from a comparison of the eggs of Asterias forbesii and Toxopneustes variagatus. Both of these eggs are semi-transparent and contain the minimum quantity of yolk required for the formation and establishment of an independent larval form. In all physical properties, except that of the size, they seem to be similar, the former having a diameter of about 0.15 mm. the latter about 0.065 mm. (text fig. 3, F and E.) In each case the ratio between volume of ground substance and quantity of stored yolk is probably the same. Both produce highly specialized larvae with long periods of development.

The eggs of Asterids may therefore be considered to constitute a series distinct from, but parallel to, that of Echinoids and Ophiurids in which an egg like that of Asterias stands at one end and an egg like that of Cribrella at or near the other.

Temperature is a factor which also considerably affects the rate of development of echinoderm eggs, and, since echinoderms develop under widely different and fluctuating temperatures,

it necessarily enters to make the Echinid-Ophiurid correlation
curve less accurate than is to be desired.

The size (diameter) of egg, which is one of the factors used
in constructing the curve, is again not the real factor involved
in the correlation. The real factor, the ratio between the vol-
umes of living substance and yolk substance of the eggs, which
it is not possible to measure or estimate, is most nearly approxi-
mated by a comparison of diameters of eggs, assuming that the
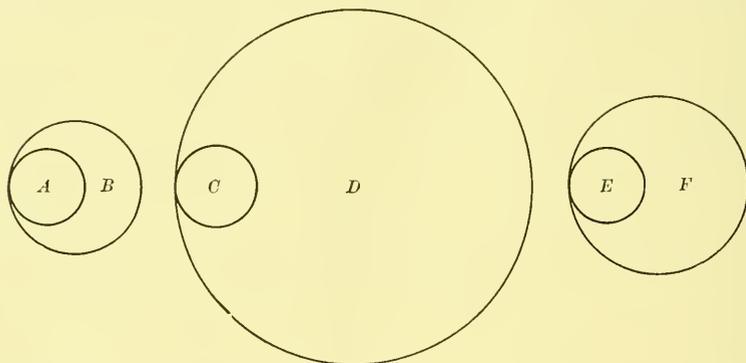
Text fig. 3 The relative sizes of the eggs of certain echinoderms are here
shown by circles.

C, the egg of Ophiocoma (of minimum size), superimposed upon D, the egg
of Ophiura (of possible maximum size), to show the relative difference in size
of the eggs of Ophiurids due to difference in yolk content.

A, the egg of Toxopneustes, and B, the egg of Mellita, brought together to
show the same for the eggs of Echinids.

E, the egg of Toxopneustes, and F, the egg of Asterias, superimposed to show
the great difference in size between eggs of the minimum size characteristic of
Echinids and Asterids.

volume of living substance in all eggs of the Echinid-Ophiurid
series is substantially the same.

Apparently a reduction of the amount of yolk material in
an egg results in prolonging the period of its development, but
there is a minimum limit to which such reduction may be car-
ried, namely; to an amount insufficient for the establishment
of those larval structures and functions necessary for the cap-
ture and digestion of plankton organisms. In the minute eggs

of Toxopneustes, Ophiocoma, Asterias, etc., such a limit has
apparently been reached; but, in the increase in yolk con-
tent and size of egg, at the other extreme, there seems to be no
limit and the eggs of certain species contain an amount of re-
serve material capable of carrying the young considerably
beyond the stage at which metamorphosis has been completed
and in which the adult organization has been attained, viz:
Solaster.
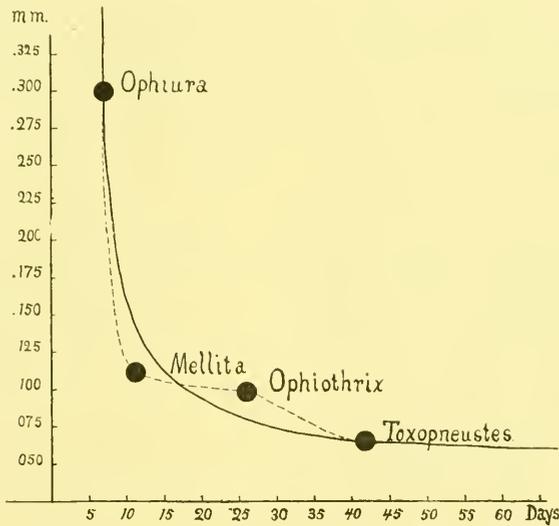
Text fig. 4 Curve intended to indicate the correlation which exists between
yolk content and size of egg, and the duration of larval development in echino-
derms.

Increase in the size of the egg, however, involves a corre-
sponding decrease in the number of eggs that can be produced
by an individual, and, when the eggs of a species become very
large and correspondingly few in number, a limit of egg-pro-
ducing capacity must finally be reached at which the extinction
of the species would be threatened, provided the normal rate
of mortality among the progeny were not also reduced. Brood-
ing of eggs and young by the parent has the effect of reducing
the mortality among offspring and it is a most interesting fact

that those echinoderms in which various peculiar methods of brooding are found, are just those which produce a comparatively small number of very large yolky eggs, viz: Cribrella, Amphiura, Cucumaria, etc.

When brooding has been established in a species a correlation between yolk content of egg and period of larval development such as that which exists in those forms which have pelagic eggs and larvae may disappear because there is then no further necessity for haste, on the part of the brooded eggs, to assume the adult condition. Protection against the dangers of the developmental period in such cases is assumed by the parent. For this reason, in practically all cases of brooded eggs, the species fails to conform to the correlation curve.

BREEDING HABITS OF OPHIURA

None of the numerous attempts which have been made to fertilize the eggs of Ophiura artificially have been successful, but it has an easy matter by following the method outlined below, to induce Ophiura to spawn in aquaria in the laboratory during its breeding season which, at Beaufort, North Carolina, extends over the months of June and July.[3]

Method. At the time of low tide about thirty adult animals are collected from among the roots of eel grass and sedimentary debris on the shallow flats of Beaufort Harbor and are placed in an aquarium jar of about ten liters capacity. In order to keep the animals under conditions as nearly normal as possible until near the time of day when spawning has been found to take place (shortly after twilight), the jar containing them is placed in running water (suspended beneath a wharf) until about sunset. The animals are then transferred to the laboratory, placed in another aquarium jar of fresh sea-water and allowed to stand undisturbed before a window.

For a time after this transfer the animals may be observed to huddle together near that side of the aquarium least illumi-

[3] The earliest date on which a set of mature eggs has been secured is June 5, the latest, July 26

nated but as dusk comes on they become active, crawl about over the bottom and attempt to climb the side of the jar. At about eight o'clock spawning begins. The spawning process is initiated by the males, but very soon after sperm has been emitted into the water the females begin to liberate eggs.

The presence of sperm, or of some substance associated with the sperm, in the water of the aquaraium containing the females seems to be the necessary stimulus to egg laying. In the presence of a greatly diluted solution of the spermatic secretion, such as may be assumed to be present under normal conditions in the open sea, it is probable that mature eggs only are extruded by the females, but in aquaria the water becomes clouded with sperm, and, as if overstimulated by this unusually large amount of sperm, the females throw out their entire content of eggs, the immature with the mature.

It is difficult fully to test this conclusion since there is no external character by which the males of Ophiura may be distinguished from females. The conclusion rests upon the observation that in those cases when it has happened that the number of males placed in an aquarium has been few, a much larger per cent of the eggs then secured has been mature than in cases in which the number of males has been large..

A female, while extruding her eggs, either stands upright, supported by the side of the aquarium, or with her body held horizontal to and high above the bottom by her strongly arched arms. While the body is thus elevated the eggs pass from the distal openings of the genital bursae into the water and slowly rise to the surface, their appearance at this time being like that of ascending streams of minute bubbles of air.

This peculiar position of the body of the female during the spawning process, (and it is sometimes assumed by the male also) has been observed in species of Ophiurid other than Ophiura. It may be accounted for as an adaptation by which these animals, which live just beneath the surface of the bottom or under conditions such that their bodies are covered for a considerable part of the time by surface debris, provide for the unobstructed passage of their eggs into the free water above, where condi-

tions favorable for development prevail.   If the females while spawning did not thus lift their bodies above the sedimentary materials by which they are normally covered, a considerable portion of the eggs might possibly become entangled in the debris and fail to reach the surface.

The fact that the spawning activities of Ophiura are limited to a definite time of the day, (at or near eight o'clock in the evening) is one for which a satisfactory explanation is wanting. The suggestion at once presents itself that the advent of darkness and the changes in temperature which take place soon after sunset, may be regarded as having had something to do in establishing the time for spawning at an early evening hour, but, from our present knowledge of conditions, the advantage to be gained by limiting the activities of spawning to this evening hour does not appear.   In this connection it should be borne in mind that Ophiura is a nocturnal species.   The coming of darkness is, therefore, a condition which awakens the animals to activities in general, not alone those activities which are connected with reproduction.   As soon, therefore, as an Ophiurid, which has its genital bursae filled with mature eggs or spermatozoa, is aroused from the torpor of the day, it is possibly incited to immediate sexual activity by stimuli arising from the ripe sexual elements.   Until the mature sexual elements have been extruded, the stimuli to sexual activity arising from them may be considered to overshadow the stimuli to all other activities.

If, however, this were a complete explanation for the observed fact that Ophiura spawns soon after darkness falls, it should be possible to induce sexually mature animals to spawn at any hour of the day simply by keeping them for a time in a dark cool room, but thus far such an experiment has failed to bring about the expected result.

SUMMARY AND CONCLUSIONS

1. The great increase in the yolk content of the egg of Ophiura brevispina, to which its large size is due, has not disturbed its early developmental processes. The manner and rate of its segmentation and the structure of the blastula remain practically the same as in the egg of Ophiocoma which is one seventy-eighth the size of the egg of Ophiura.

2. The coelomic structures of Ophiura take their morphological origins at three widely separated parts of the larva which might be taken to indicate that the organization of the egg of Ophiura is fundamentally different from that of echinoderms like Arbacia, Strongylocentrotus, etc., in which the mesodermal material has been shown to be localized in the egg, at the time of fertilization, in a middle horizontal zone and carried out during gastrulation in one mass to the end of the archenteron, there and then to be distributed as five coelomic structures. Assuming, however, that the eggs of all echinoderms are essentially alike in fundamental structure and promorphological differentiations, the peculiarities shown by Ophiura, and all other species having large yolky eggs, in the manner and place of appearance of their coelomic anlages, may be satisfactorily explained on the ground that each mesodermal organ is to be referred back in all echinoderms to a definite substance formed and localized in a definite part of the egg, and that these substances, which ordinarily flow together when fertilization takes place, are prevented from so fusing in large yolky eggs by the mass of inert yolk material with which such eggs are densely packed.

3. The yolk material of the egg influences its later development by mechanically interfering with and hindering intra- and intercellular movements. These effects are clearly apparent in the blastula of Ophiura.

4. The early gastrula of Ophiura responds to the presence of this large amount of yolk material, and its inhibitory effect upon developmental processes, by extruding the greater part of the yolk substance of each of its cells into the segmentation and arch-

enteric cavities and thus frees itself from the burden of constantly manipulating the yolk.

5. A study of the literature of echinoderm embryology indicates that some process of yolk segregation probably takes place during the development of all yolky eggs, but there is such a lack of conformity in the processes found in different species as to indicate that the process peculiar to each species has been independently acquired—that there is no genetic continuity in processes of yolk segregation found among echinoderms.

6. A close correlation between the volume of yolk material of an egg and the period of its larval development seems to exist among echinoderms.

7. The spawning activities of Ophiura brevispina take place at or near eight o'clock p.m. with the advent of darkness. They are to be correlated in part at least with the nocturnal habits of the species.

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1 Median section of a blastula of Ophiura brevispina in which the mesenchyme is differentiating. Fixation in sublimate-acetic . Cell outlines are not evident, and the yolk material has been dissolved and removed during dehydration, but the former positions of the yolk spherules are indicated by minute vacuoles.

2 Part of a cross section of a blastula, fixed in osmic Müller solutions, showing resting and dividing cells containing yolk spherules.

3 Part of a tangential section of a blastula, fixed in osmic-Müller solutions, showing resting and dividing cells.

1

2

3
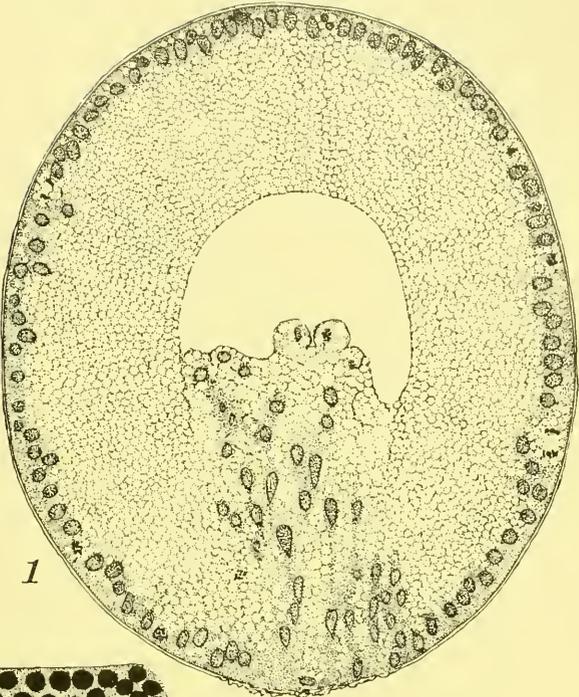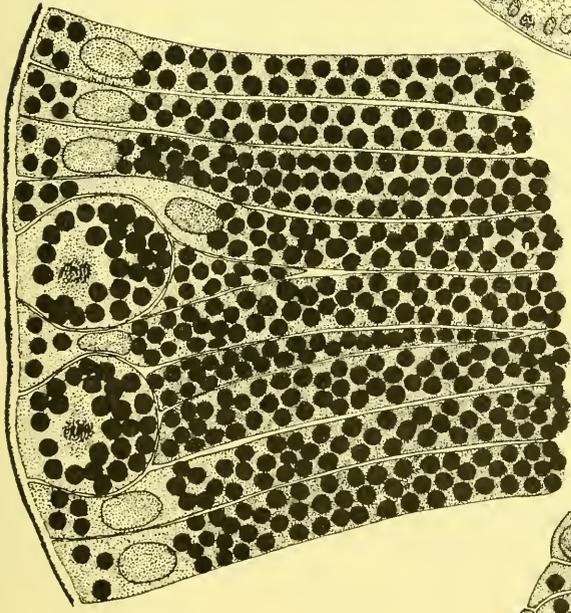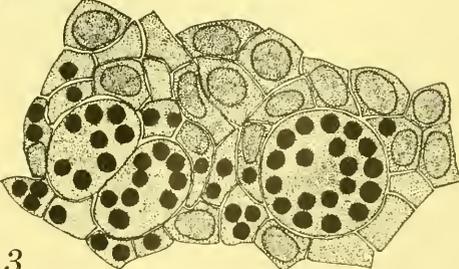
4 Drawing by C. Kellner of a reconstruction of a larva of Ophiura, ventral view, at the stage in which the coelom and alimentary canal have been differentiated. Above and to the right and left of the mouth opening, the small right anterior enterocoele and the larger left anterior enterocoele are shown. These enterocoeles are somewhat obscured by the five lobed hydrocoele which, at this stage, still retains its connection with the left posterior enterocoele. The gut lies between the left and right posterior coelomic pouches and is in wide communication with the former. The right posterior coelom (epigastric) still retains its connection with, and shows its origin from, the ectoderm at a point to the right of the blastopore scar. No attempt has been made to show the large mass of yolk material and the mesenchyme cells which practically fill the blastocoele.

449
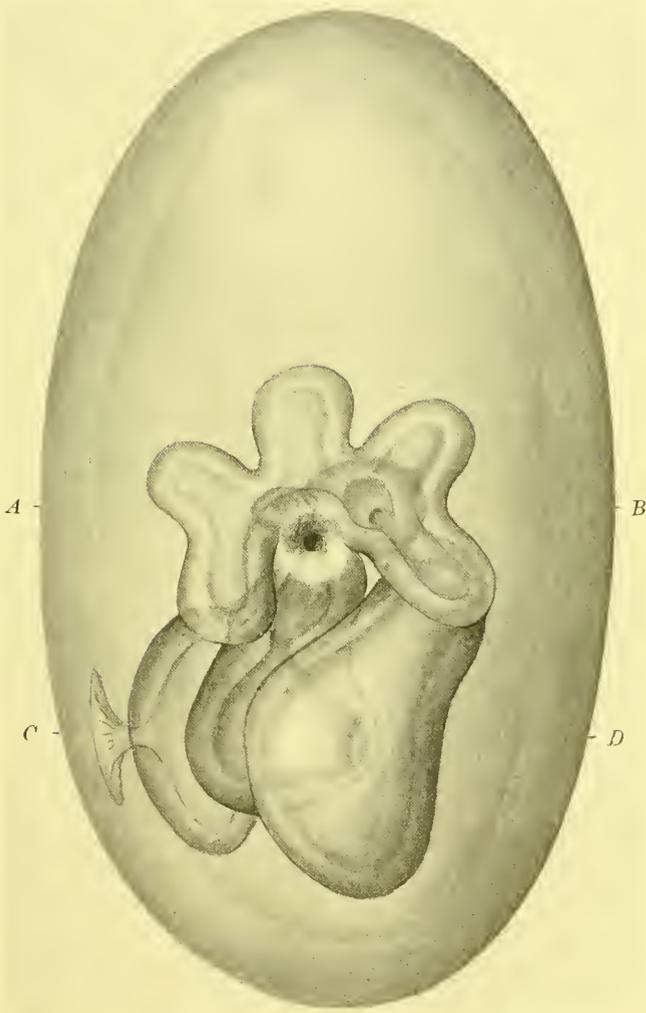
5 Cross section of the larva in the stage shown in figure 4 in a plane indicated on figure 4 by the dotted line A-B.

6 Cross section of the same larva in a plane indicated on figure 4 by the dotted line C–D.

ABBREVIATIONS

Bp, Point at which the blastopore closed.

G, Gut.

LAE, Left anterior enterocoele.

LPE, Left posterior enterocoele.

M, Stomodeum.

RAE, Right anterior enterocoele.

RPE, Right posterior enterocoele.

R2, Anlage of radial canal No. 2.

R4, Anlage of radial canal No. 4.
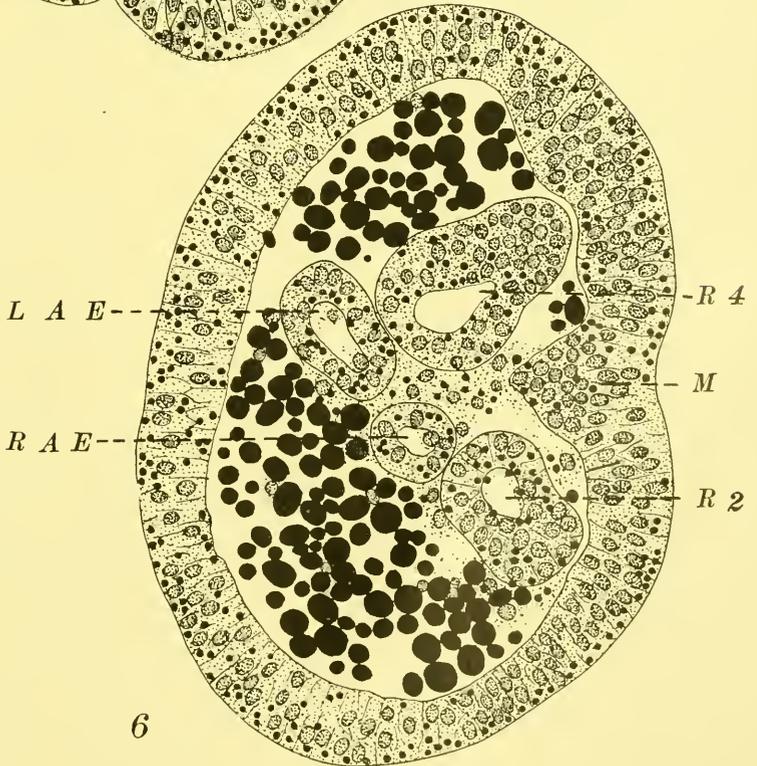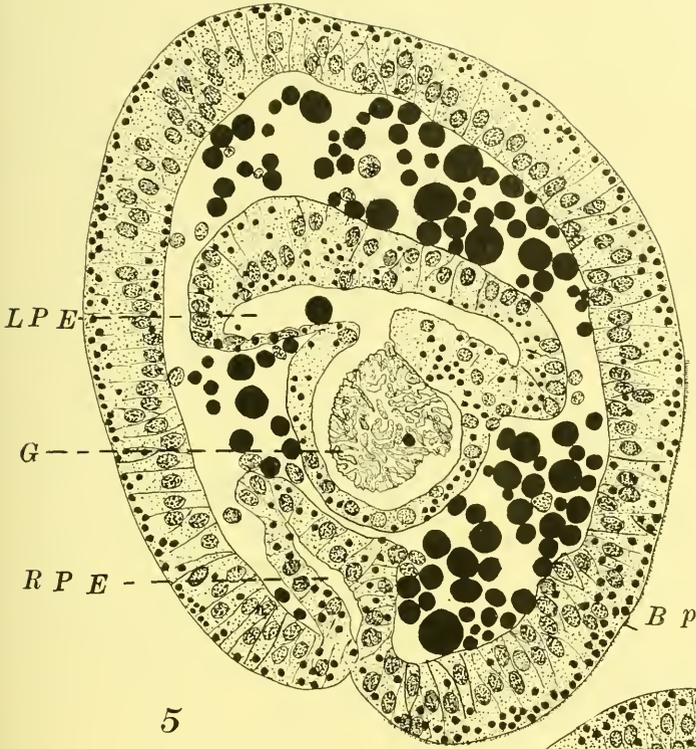
THE DEVELOPMENT OF PARAVORTEX GEMELLIPARA
(GRAFFILLA GEMELLIPARA LINTON)

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SIXTEEN TEXT FIGURES AND NINE PLATES

CONTENTS

I. INTRODUCTION

1. Determination of the species

In the Brooks Memorial Volume of The Journal of Experimental Zoology, vol. 9, 1910, Prof. Edwin Linton described a new species of Rhabdocoele Turbellarian which he had discovered living in the ribbed mussel, Modiolus demissus. He referred it with some hesitation to the genus Graffilla (Jhering). Von Jhering ('80) in establishing the genus Graffilla characterized it in the following words:

Pharynx nach Art des Mesostomeenschlundes aus Muskelfasern und eingelagerten Bindegewebszellen bestehend, nicht in einer Tasche gelegen, sondern direkt mit dem Munde zusammenhängend. Bursa copulatrix und Receptaculum seminis durch ein Organ vertreten. Ovarien langgestreckt, bandfömig, Dotterstöcke sehr reich verästelt.

Most important in the determination of the genus in which Linton's Turbellarian is to be placed is the question of the receptaculum seminis. Von Jhering in his figures of Graffilla muricicola indicates that two reservoirs are connected with the atrium (called by him the 'uterus'). The more anterior of these is the vesticulum seminalis which bears a short conical penis on its lower surface and projecting into the atrium. The receptaculum seminis lies just posterior to the seminal vesicle, and likewise dorsal to the atrium. It s to be noted that the latter is not merely a dorsal lobe of the atrium but is a distinct spherical reservoir, fully as large as the seminal

vesicle, and connected with the atrium by a slender duct of
a length about equal to its own diameter. This canal as de-
scribed by von Graff for Graffilla buccinicola is shorter but none
the less definite. It is therefore characteristic of the genus
Graffilla that the seminal receptacle is not sessile upon the atrium
but connected with it by a canal. An examination o᾿ the
Turbellarian discovered by Linton reveals no such reservoir.

This American species may be described as a Vorticid Rhab-
docoele occasionally reaching 2 mm. in length and 0.80 mm. in
diameter at its widest part; elongated ovoid in form while at
rest, young specimens nearly linear when moving rapidly, pos-
terior half of body somewhat broader, anterior quarter more
flattened than central and posterior portion which is nearly
cylindrical; ends bluntly pointed, the posterior often being the
more acute; white with yellowish tinge, semi-transparent;
body contractile and extremely flexible; crawls on bottom some-
times in a straight line, often performing circus movements;
sometimes swims freely in water, revolving about its longitudinal
axis and so follows a spiral course; pharynx sub-globose (dolii-
form) opening anteriorly into a small vestibule leading to the
mouth; the latter is ventral but so close to the anterior end that
it is directed forward; oesophagus not distinct from the spindle-
shaped intestine into which it merges; intestine extends nearly
to the posterior end, lies in upper half of body, walls thick,
composed of large cells elongated toward the lumen; two black
reniform eyes above the pharynx and anterior to the well devel-
oped two-lobed brain. Genital pore ventral at the end of the
anterior third of the body, opening into a large atrium; promi-
nent pear-shaped seminal vesicle carries at its lower end a mus-
cular penis (without chitinous parts) which lies in a very small
atrium masculinum; atrium produced posteriorly and dorsally
into a pouch which acts as a bursa copulatrix; distensible an-
trum femininum extends posteriorly from the middle of the
posterior wall of the atrium; antrum indistinctly forked at its
posterior end to form two rudimentary oviducts, but usually
so distended with spermatozoa (thus acting as bursa seminalis)
that no forking is evident; two large horn-shaped ovaries one

on either side below the median frontal plane, extending from anterior fifth to middle of the body; two extensively branched and anastomosed vitellaria; ovaries and vitellaria not joined but closely apposed at their proximal ends where they meet the rudimentary oviducts; testes paired, situated one on each side just laterally and posteriorly to the pharynx; vasa deferentia short, arising from the inner posterior margins of the testes and opening into the seminal vesicle on its anterior surface; bursa seminalis lacking; viviparous, the young developing, usually in pairs, inside capsules of which as many as forty may be present at once in the mesenchyme of the mother; young break out of capsules and swim about in parent until they emerge through body wall or intestine; rolled up capsule shells remain in the 'mesenchyme.

Paul Hallez has given ('09) a detailed account of his observations on Paravortex cardii, a Rhabdocoele belonging to a genus closely related to Graffilla. The resemblance between Linton's and Hallez's species, as will be indicated below, is very striking.

The genus Paravortex was established by Wahl ('06) for a species of Turbellarian which is parasitic in the alimentary canal of Scrobicularia tenuis and S. piperata. According to Wahl it was discovered and inaccurately figured by Villot. Von Graff later called this parasite Macrostomum scrobiculariae, but Wahl asserted that it was worthy to be placed in a new genus. This he called Paravortex. He also found von Graff's Provortex tellinae to be identical with it.

Wahl ('06) briefly described the new genus Paravortex in the following words:

"Dalyelliide mit am Vorderende des Körpers gelegenem Pharynx, paarigen Keimstöcken, verzweiten Dotterstöcken, rundlichen Hoden und ventral vor der Körpermitte gelegener Geschlechtsöffnung."

In the same paper Wahl characterizes the genus Graffilla thus:

"Dalyelliide mit am Vorderende des Körpers gelegenem Pharynx, paarigen Keimstöcken von der Form gewunderen Bänder und davon getrenntren Dotterstöcken. Geschlechtsöffnung mittelständig, Hoden schlauchartig."

Concerning the presence or absence of a bursa seminalis Wahl says nothing, but his figures of the reproductive organs show very clearly that no such reservoir exists in Paravortex. We have already seen that this organ is characteristic of Graffilla.

According to Wahl, the genus Paravortex is distinguished from Graffilla, since its members have the genital pore situated in front of the middle of the body while in Graffilla this opening is at or behind the middle. The ovaries of Paravortex are club-shaped, while those of Graffilla are sinuous and band-shaped. The testes of Paravortex are nearly spherical; those of Graffilla appear bottle-shaped.

Von Graff gives ('08) a key to the Family Graffillidae, which is translated in part below:

A. With two germo-vitellaria and two compact, unlobed testes.
    1. Pharynx well developed, germo-vitellaria not lobed.........Vejdovskya
    2. Pharynx small, germo-vitellaria lobed in the form of a hand. .Paravortex
B. With two ovaries and two vitellaria.
    1. Ovaries club-shaped, of normal size.
        a. Vitellaria long and unbranched.
            1. Testes paired, genital pore on ventral surface....Paravortex
            2 Testis single, genital pore at or near the posterior end
                    ....Collastoma
        b. Vitellaria branched (Testes paired, genital pore near posterior
            end) .... Umagilla
    2. Ovaries of different form, vitellaria branched or lobed.
        a. Ovaries abnormally long cylindrical cords, vitellaria lobed in
            form of a hand, genital pore central...................Graffilla
        b. Ovaries and vitellaria hand-shaped, genital pore at the posterior
            end.. ....Syndesmis

Thus it is seen that, according to von Graff, the genera Paravortex and Graffilla differ in that the former has two hand-shaped germo-vitellaria, i.e., the ovary and the branched vitellarium of each side unite at their proximal ends to form a single stalk which joins the oviduct. Graffilla, on the other hand, has two extremely long cylindrical ovaries which are not thus joined to the vitellaria; the latter, however, are branched.

Von Graff in his key did not refer to the receptaculum seminis, nor does he in the description of the genera dwell particularly upon this point. Nevertheless, while he fails to mention the organ in speaking of Paravortex, he notes that all the species

belonging to the genus Graffilla are furnished with "eine mächtige Bursa seminalis" (receptaculum seminis.)

In order to classify Paravortex cardii Hallez ('09) presents the following table which brings out more clearly, as I think, the generic differences between Paravortex and Graffilla.

1. A single ovary. Body more or less flattened......................(2)
   Ovaries and vitellaria paired.................................(3)
2. Ovary large, irregularly lobed. Genital pore posterior.......Anaplodium
   Ovary small. Genital pore at the beginning of the last quarter of
   the body...Didymorchis
3. Vitellaria unbranched.....................................Collastoma
   Vitellaria branched or reticulated................................(4)
4. Genital pore posterior.. (5)
   Genital pore ventral in the first half of the body.................(6)
5. Intestine lobed. A pharynx..............................Syndesmis
   Intestine straight. No pharynx in adult....................Fecampia
6. A bursa seminalis..Graffilla
   No bursa seminalis...... ....Paravortex

From Hallez's key it appears that there is a disagreement as to whether the ovaries and vitellaria in Paravortex should be considered as one organ. Von Graff asserts that they are united, while Hallez considers them separate. From his description of P. cardii it is evident that Hallez is correct.

In comparing the genera Paravortex and Graffilla Hallex says, "Les genres Graffilla and Paravortex ont entre eux des affinités très étroites. La seule différence vraiment importante qui existe entre ces deux genres c'est l'absence chez Paravortex de la bours séminale qui se recontre dans toutes les espèces de Graffilla."

From a consideration of the several descriptions and keys quoted above one may summarise the distinction between the two genera Graffilla and Paravortex in the following manner:

Graffilla	Paravortex
1. Two extremely long cylindrical ovaries.	1. Two club-shaped ovaries.
2 Genital pore ventral, not anterior to the middle of the body.	2. Genital pore ventral, anterior to the middle of the body.
3. A large and distinct bursa seminalis (receptaculum seminis).	3. Bursa seminalis lacking.
4. No vestibule between pharynx and mouth.	4. Vestibule.

It is therefore obvious, since it agrees in all four of these characteristics, that Linton's species must be referred to the genus Paravortex. It now remains to determine wherein, if at all, the American species differs from the two found in Europe. Of Paravortex cardii Hallez gave a detailed description which, translated, is as follows:

"Vorticid provided with two ovaries, two reticulated and anastomosing vitellogenous glands and two spherical testes; bursa seminalis lacking. Genital pore ventral, situated close behind the pharynx, at the end of the first quarter of the body. Male copulatory organ muscular, without chitinous parts and bearing two lobes furnished with papillae. Pharynx doliiform. Mouth ventral, near the anterior end of the body. Body cylindrical, thinner at the anterior end, uniformly ciliated, white and with a slight yellowish tinge, transparent, without rhabdites, very contractile, ordinarily bent in an arc and turning about in one spot so as to describe a circle. Two black reniform eyes above the pharynx. Maximum length, 1 mm.; diameter 0.3–0.4 mm. Viviparous. Numerous soft-shelled capsules (up to forty) each enclosing one to four embryos and distributed through the connective tissue. Empty and rolled-up shells remaining in the body of the mother. Lives in the stomach of Cardium edule."

Linton's species and P. cardii are closely similar both in structure and habits. Both have essentially the same color and the same shape of body. Both have similar digestive, sensory and glandular organs; both give birth to living young which develop in capsules within the mother's body; both show the same peculiar movements when taken from their host and placed in sea water.

The two species differ, however, in that the American form attains twice the size of P. cardii; the genital pore is situated farther posteriorly and the ovaries are longer in the latter; an atrial canal in P. cardii leads from the dorsal part of the atrium backward to the antrum femininum, while in the American species there is no distinct canal but rather the antrum femininum extends backward from the middle of the posterior

atrial surface and its opening into the latter is strongly constricted by a sphincter muscle; the openings of the shell glands in P. cardii are distributed along the entire ventral wall of the atrial canal and antrum femininum, while in Linton's species they all open at the anterior end of the antrum just back of the atrium; the vitello-oviducts of P. cardii are the longer. Linton's species lives as a commensal in the mantle cavity of the ribbed mussel, Modiolus demissus; P. cardii is parasitic in the stomach of Cardium edule.

Linton's species resembles Paravortex scrobicularia rather than P. cardii in the form of the ovaries, i.e., they are elongated in the first two and shorter in P. cardii. In form of antrum femininum and lack of an atrial canal Linton's species and P. scrobicularia are similar, but the latter, on the other hand, is similar to P. cardii rather than to the American form in that the atrium does not project dorsally beyond the opening of the antrum.

Since Linton's species obviously differs from the European forms, his specific name gemellipara is to be retained. Its applicability is owing to the fact that ninety-five per cent of the capsules contain two embryos.

2. Historical

While the anatomy and taxonomy of the Rhabdocoele Turbellaria have received considerable attention, only a few works have appeared dealing with their embryology. Hallez ('78) published the results of his observations upon the winter eggs of various Turbellaria. Since the young are enclosed within a hard-shelled capsule, the material was so difficult to study that he characterized these results as insufficient. In 1887 he did further work upon the fresh water Dendrocoeles.

In 1903 Caullery and Mesnil described the development of certain species of Fecampia, Rhabdocoeles parasitic in certain Crustacea.

One of the most complete and noteworthy contributions to the embryology of this group is that of Bresslau. In 1904 he published the results of his research, begun in 1898, upon the embryology of four species of the family Mesostomidae, namely

Mesostomum ehrenbergi, M. productum, M. lingua, Bothro-
mesostomum personatum, and of one Alloiocoele, Plagiostomum
girardi.

Another remarkable contribution to Rhabdocoele develop-
ment is the section devoted to embryology by P. Hallez in his
later work ('08), to which we have already referred, upon Para-
vortex cardii, a Rhabdocoele parasitic in the stomach and
intestine of the mollusc Cardium edule. He therein reinterprets
the significance of certain figures published by Caullery and
Mesnil. As a result of the behavior of the ectoderm and ento-
derm cells to be described in this paper some of Hallez's figures
in turn suggest an interpretation differing from his own. The
condition at corresponding stages in the development of Para-
vortex cardii and P. gemellipara shows in many respects a
striking similarity. In the formation of the ectoderm and
the manner in which the vitellarial yolk enters the embryo,
however, the process in P. gemellipara differs distinctly from
that described for P. cardii. The ectoderm differentiation
agrees essentially with that found by Bresslau in Mesostomum
ehrenbergi. On the other hand, though the end result is the
same, the manner in which the yolk is taken into the embryo
of P. gemellipara presents, so far as the literature studied has
revealed, marked variations from that described for any animal.

While Linton made no serious attempt to discover the origin
of the twins in Paravortex gemellipara, he suggests that the
twin condition might be due to a process of polyembryony.
In order to settle this point J. T. Patterson in the summer of
1911 continued the study of the worm at the Woods Hole Labora-
tory. The results of his work appeared in 1912.

The purpose of the present paper is to describe the main
features in the behavior and development of Paravortex gemel-
lipara. Particular attention is to be paid to the processes of
nutrition as taking place in all stages from the oocyte to the
free-swimming worm, comparing them with homologous pro-
cesses observed by investigators working on other forms.

It is a pleasure to express here my sincere appreciation of the
kind assistance offered me in all stages of this research by Dr.
Wesley R. Coe.

II. BIOLOGY, MATERIAL AND METHODS

Biology and material

Paravortex gemellipara was stated by Linton to be a commensal living on the gills of Modiolus demissus, but Patterson concluded that the worm lives primarily in the kidney, although he thinks it likely that many individuals escape and may then be found in the mantle cavity and on the gills. Patterson opened two dozen mussels, using care not to injure the tissues, When washed in water no parasites were found, but after tearing the kidneys of these same mussels apart, he secured thirty-eight specimens. Hallez ('09) found P. cardii in the stomach and intestine of Cardium edule. So, thinking that in attempting to dissect the kidney, Patterson might have cut the alimentary canal, I made several attempts between May and September to discover the worm in the stomach, intestine, kidney and liver.

After the adductor muscles alone had been carefully cut and the valves forced apart eight to sixteen mussels were vigorously washed in sea water. If more be opened at once the sediment renders the Turbellaria difficult to detect. The worms were allowed to settle to the bottom, and, the better to see the bottom of the dish, most of the water was slowly decanted. The vessel was then placed upon a dark surface in good light. In looking for the very young a hand lens was found useful. Next the stomachs of the same mussels were carefully slit open with dissecting scissors and the interior washed in another dish by means of jets from a pipette. Water was also forced through the intestine in the same manner. Finally, in a third vessel, the same mussels were again washed after tearing apart all the tissues, thus insuring the breaking open of the kidney, an organ extremely difficult to distinguish and dissect without disturbing other tissues.

It was found that two hundred thirty-seven mussels collected at various times during the year yielded with the first washing one hundred eleven specimens of Paravortex, with the second two and with the third six. In one instance thirty-seven worms

were removed from eight mussels after the adductors had been cut, and only a single one when the viscera had been dissected. Considering the numerous crevices between the gills, visceral mass and mantle it seems very improbable that those worms which were not dislodged during the first washing were within the alimentary canal or kidney, but became freed from the mantle cavity in the two subsequent rinsings. The evidence points to the latter as being the usual abode of Paravortex gemellipara.

The number of worms obtained from different lots of mussels varies immensely. Patterson is of the opinion that there is a periodicity in the reproductive activity of this animal, but it is probable that the difficulty which he sometimes found in obtaining specimens was merely due to the irregularity of their occurrence. It has often been observed that one pailful of mussels may yield an abundance of worms, while another collected in a few hours and from the same mussel bed may produce very few. The conclusion is that only a portion of the mussels in the bed contain commensals. The success in obtaining specimens, therefore, depends upon the selection of the proper mussels.

Although usually no accurate count of the number of worms taken from given numbers of molluscs has been kept, a great variation is known to occur in their distribution. In the vicinity of New Haven Paravortex is abundant in certain mussel beds and almost entirely wanting in others. Any situation between high and low tide marks seems to be as favorable as another. Not always do those mussels which receive the most sunlight, even in winter, yield the greatest number of worms. In December, beds which were exposed to bleak winds sometimes gave better results than others which were fastened to rocks in sheltered coves with southern exposure.

Although, doubtless on account of increased metabolic activity and hence more prolific reproduction, Paravortex is more abundant in warm weather, still specimens may be obtained without difficulty at all seasons. I have collected them during every month of the year. Hallez asserts that P. cardii is as abundant

in late autumn as in summer at Le Portel, nor does he find them less numerous in December and April. The greatest number of P. gemellipara obtained at New Haven was collected on November 9, 1912, when about 175 mussels yielded 310 worms. Only those easily detected with the naked eye were taken.

Linton states that he found this species negatively phototropic; that on leaving a dish containing specimens in strong light the worms were afterward found to have collected on the side opposite the source of light. My observations, on the other hand, point to a neutral reaction on the part of the older worms and a positive reaction of the younger. The adults are as often found on the light side of the dish as on the dark. Their observed tendency to secrete themselves beneath debris on the bottom of the dish was at first interpreted to signify that they were reacting negatively to light, but when it was ascertained that in a dish lacking such debris these older individuals seemed neutral it was concluded that thigmotropism was responsible for their hiding reactions. It has been noticed that an adult worm, upon coming in contact with a piece of mussel gill or other tissue, often crawls along in contact with it.

The young worms, on the other hand, are positively phototropic. If a number of them be placed in a watch glass and the latter then rapidly rotated so as to cause the collection of the worms at the center, as the water comes to rest they immediately swim vigorously toward the source of light. Only daylight was used. This experiment was made more striking by the introduction into the watch glass of several specimens of Aphanostoma. The latter, being negatively phototropic, as rapidly crawled to the darker side of the glass. Repeated trials were followed by the same reactions, the two groups of worms separating at the center and progressing in opposite directions.

There is a marked tendency on the part of the young worms to crawl up the side of the dish above the water line; death often resulted.

One can conceive that thigmotropism may be of value in causing the older worms to remain in the mussels. Linton

suggested that negative reaction to light brought about this result, but as explained above it is extremely unlikely that the adults are thus negatively reactive to daylight. The marked positive reaction of the young specimens is probably responsible to some extent for their leaving the host within which they develop and so gives them an opportunity to enter other mussels. In the fulfillment of this end the negative geotropism of these young worms may also be of service; it would tend to cause a migration upward toward the source of light.

When removed from their hosts the healthy worms of medium size glide along the bottom of the dish at the rate of about one millimeter per second. Linton noted that the course in one direction was not long held, but often changed from one side to the other following a turning movement of the anterior end of the animal. Frequently these older worms halt and turn rapidly about for several revolutions in a circle whose diameter does not exceed the length of the individual (fig. 2). Hallez noted this circus movement as being extremely frequent in the case of Paravortex cardii.

The young worms move with greater rapidity for their size and in a much more direct course than the older invididuals. Furthermore they follow one direction often for several inches, only deviating in order to avoid obstacles. As already stated this course is consistently toward the light source. Rarely does a young worm perform the circus movement.

It is interesting to note in passing that Paravortex gemellipara like other Turbellaria observed by von Graff, is itself subject to parasitism. Frequently while studying living worms under the microscope numbers of an hypotrichous Infusorian are seen moving over them. One worm was so completely covered with them that, when the cover slip was pressed, these Infusoria became separated in such a way as to give the impression that the epithelium of the worm was peeling off. The hypotrichs, being ciliated only on one side, and closely approximating the epithelial cells in size, appeared strikingly similar to them.

Methods

Observations made on the living worm slightly compressed beneath a cover slip enable one to make out the genital pore, ovaries, testes, seminal vesicle and vitellaria. But for the genital ducts this method proved unsatisfactory. In one instance a mass of spermatozoa was distinguished in the atrium of a specimen which lay with its ventral surface up.

The most reliable and complete data were obtained from sections of the worm. Of specimens killed in corrosive sublimate, Zenker's fluid and strong Flemming solution and subsequently stained with iron haematoxylin, those killed in Flemming solution gave the most satisfactory results, both as to preservation and stain. In most cases Orange G was used as a counter stain.

Material fixed in corrosive-acetic and stained in Ehrlich's haematoxylin was excellent for the study of tissues in which the exact condition of the chromatin was of minor importance. Nuclei, as a whole, show much more plainly when treated by this method than when iron haematoxylin is used. Here again orange G was used as a counter stain. In connection with the study of sectioned material living embryos of various stages, removed by pressure from the mother, furnished checks on many points. A few useful total mounts were prepared.

III. ANATOMY OF THE ADULT REPRODUCTIVE ORGANS

The male organs

Like the related forms described by Bresslau, Hallez, von Jhering and von Böhmig, Paravortex is protandrous. In half-grown individuals the male organs are well developed. The testes lie one on either side of the body close behind the pharynx (fig. 3). A large seminal vesicle lies just above the genital pore which opens in the midline of the ventral surface about one-third the distance from the anterior end of the body. Two short vasa deferentia lead from the inner posterior margins of the testes to the seminal vesicle. A common atrium intervenes between the seminal vesicle and ventral body wall in such a way that the plug-like penis, situated in the small atrium

masculinum at the lower end of the seminal vesicle, is directed through the common atrium toward the genital pore (text fig. 1). In no worm whose testes had become functional was the seminal vesicle found empty; usually it is distended with a mass of spermatozoa. All these organs are surrounded by a cellular wall.
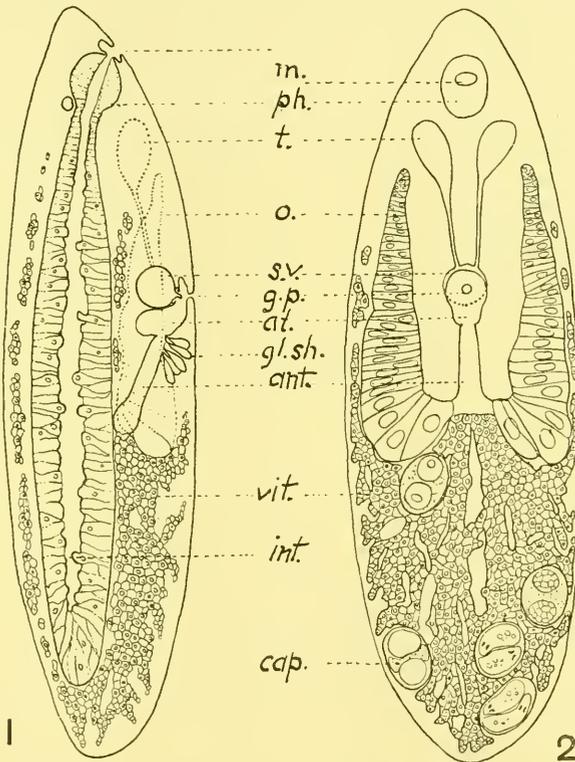
Text fig. 1. Sagittal section of Paravortex gemellipara. × 167.

Text fig. 2 Optical frontal section from below. *ant.*, antrum; *at.*, atrium; *cap.*, capsules; *g.p.*, genital pore; *int.*, intestine; *m.*, mouth; *o*, ovary: *ph.*, pharynx; *s.v.*, seminal vesicle; *gl.sh.*, shell glands; *t*, testis; *vit.*, vitellarium. × 167.

In half-grown worms the male reproductive organs have reached maturity; after this period the testes with their ducts undergo partial degeneration. Patterson states that they are seldom found in large individuals, but I have seen them in every specimen examined.

Mitoses in the process of spermatogenesis are most readily observed in the younger worms; probably few spermatozoa are matured after the female organs begin to function. There is evidence, however, that this may happen. One series of sections of an old worm cut transversely shows a considerable number of apparently mature spermatozoa in the posterior dorsal portion of the testes and in the sperm ducts which can be traced for some distance backward toward the seminal vesicle.

The form of the spermatozoon is such as might, from its structure in nearly related animals, be expected. Von Graff ('02) figures and describes the spermatozoon of Graffilla buccinicola as 0.6 mm. in length with an oval head and a flagellum about twenty-five times as long. Von Jhering ('80) found in Graffilla muricicola spermatozoa some of which exhibited bifurcated heads with straight prongs. In others he was unable to observe this forked condition, and suggested that it might not be universal. Von Böhmig describes for the same species spermatozoa with heads thus bifurcated but with the prongs curved backward.

The spermatozoon of Paravortex gemellipara is composed of an unbifurcated head 0.01 mm. in length and an extremely slender cylindrical flagellum 0.10 mm. long (fig. 5). The head is slightly curved at the tip. The living spermatozoa are motionless while in the seminal vesicle, but when liberated in sea water they exhibit writhing movements. Evidently this behavior is not the normal mode of progression which takes place in the atrium and antrum, for no progress is made through the water; the spermatozoa merely twist about in one spot. Frequently they remain motionless in the water. Presumably the fluid inside the female passages ordinarily stimulates them to action.

The seminal vesicle in Paravortex gemellipara, as in the European species of Graffilla described by the German authors, functions not only as a reservoir but also as a 'ripening chamber.' Beside mature spermatozoa, many spermatids are usually found. Figure 48 was drawn from a living worm which had been compressed beneath a cover slip. In this seminal vesicle there were

about twenty-five spermatids at a stage so young that the differentiation of the flagellum had but just begun. The nuclei could readily be seen in those cells which lay near the surface. Numerous spermatozoa were present as well. On another occasion a living animal while under observation on a slide became crushed. Among the mesenchyme cells which escaped from the body were many spermatids. Each cell had the form of a thick concavo-convex lens attached to the flagellum at such an angle as to give the spermatid the appearance of a ladle. These spermatids are always present in the seminal vesicles of worms whose ovaries have begun to set eggs free, but in the very young in which the testes have just commenced to function all the sperm cells are mature on leaving the testis.

Female reproductive organs

As the male organs degenerate those of the female apparatus undergo rapid development. By the time the animal is two-thirds grown the ovaries and vitellaria are prominent. The ovaries are situated one on either side of the body below the median horizontal plane. At the small anterior end of each ovary, which lies just posterior and slightly dorsal to the testis of the same side, numerous oogonia are crowded irregularly together. Cell membranes can be distinguished except between the first two rows of nuclei. In some specimens a terminal filament passes forward from the tip of the ovary and joins an indistinct mass of cells on the posterior ventral surface of the testis. As will be seen in the part of this paper which treats the development of the reproductive glands, the ovary and testis arise from a common mass of undifferentiated cells, and later usually become separated. Where in later stages this strand of cells remains between the two glands it is regarded as a bridge which has failed to break down.

Striking spireme figures are to be observed in the oogonia. A short distance back from the anterior end of the ovary, however, only cells with resting nuclei occur. These are primary oocytes. Beyond this point the flattened cells are packed

closely together one behind another, as Patterson suggests, like
a "rouleau of coins." The ovary increases greatly in diameter
toward its posterior end. At about the mid-length of the ani-
mal it turns inward to meet the oviduct (figs. 3, 4; text fig. 2).

The enormous paired vitellaria discharge their contents into
the same ducts as the ovaries. Each vitellarium sends branches
anteriorly and posteriorly. At the height of their development
the numerous anastomosed branches completely surround the
intestine, occupying practically the whole region between it
and the body wall (fig. 3). The anterior lobes lie above the
ovaries and intestine; in extreme cases a few branches grow
forward to a point above the brain. Single rows of yolk cells
constitute the ultimate lobes. Indeed, individual cells fre-
quently lie isolated in the parenchyma.

So closely do the vitellaria press upon the ovaries at the
posterior ends of the latter and for some distance along their
dorsal surfaces that the two organs occasionally appear to be
joined into a single germo-vitellarium on each side of the body.
As a matter of fact, however, if they join at all it is only at the
point where they meet the oviduct—or better, the 'vitello-
oviduct.' The stalks of the two vitellaria, as seen in figure 4,
are separated only by a thin partition of mesenchyme. No
cellular wall has been observed between the ends of the oviducts
and the vitellaria. As shown in this figure, yolk cells often
appear in the upper end of the antrum, indicating that they
are likely to be liberated by any slight pressure in this region.

Posterior to the seminal vesicle a dorsal diverticulum of the
atrium commune is considered by Patterson to be the degen-
erate seminal receptacle, although he found no spermatozoa
in the sac. In several of our preparations it contains a mass
of spermatozoa (fig. 9) which so distend it that the top is on
a level with that of the seminal vesicle. This organ is rather
to be regarded as a bursa copulatrix than as a receptaculum
seminis; the spermatozoa, during copulation, are first received
in this dorsal pouch of the atrium commune and afterwards pass
into the antrum femininum. The atrium is lined internally
with a high columnar epithelium, externally by a single layer

of flat cells (fig. 51).   A comparison of text figure 1 with Hallez's
brings out the difference between the two species as regards the
shape of the atrium, atrial canal and antrum femininum.   In
P. cardii the atrium rises only a very little above the opening
into the atrial canal, while in P. gemellipara the antrum opens
into the atrium at the middle of its posterior surface.   It was
stated in the introduction that the lack of a bursa seminalis
distinguishes the genus Paravortex from Graffilla.

The delicate-walled antrum femininum (text figs. 1, 2) ex-
tends from the atrium commune posteriorly and dorsally to
a point beneath the intestine.   Strong sphincter muscle fibers
constrict the opening between antrum and atrium.   So delicate
are the walls that only occasionally can the cells be made out.
Figure 4, referred to above, was drawn from a horizontally
sectioned animal which showed the walls of the antrum ex-
ceptionally well.   The section chosen included only the upper
end of the antrum; the rest of the structure must be conceived
as lying below the plane of the paper.   No marked bifurcation
is evident here on account of a distention with yolk cells; the
ends of the vitellaria border the whole posterior surface of the
antrum, while the terminal egg in each ovary presses inward
against its outer angle.   No uterus homologous to that found
in many Turbellaria is present in Paravortex.   In this regard
the following extract from von Böhmig's contribution to Bronn's
Klassen und Ordnungen des Thier-Reichs is of interest:

"Bei allen diesen, dient, wie u. a. schon Böhmig für Graffilla
muricicola und Vedjovsky für Phaenocora hervorgehoben haben,
das Atrium commune oder Antrum femininum als Uterus   .   .   .
zugeschrieben werden."

Beneath the antrum and posterior to it are groups of single-
celled shell glands.   Each contains a prominent nucleus and a
considerable amount of granular cytoplasm (figs. 3, 9).   Al-
though it is difficult to determine the exact point at which these
glands are attached to the reproductive tract, they all converge
toward the lower end of the antrum near its union with the
atrium.   As noted above, this character helps to distinguish

P. gemellipara from P. cardii in which the shell glands open along the entire central surface of the antrum.

Although no one has recorded observations of Graffillidae in the act of copulation, it is inferred that pairing occurs. During the process, as described for other Turbellaria, masses of spermatozoa from the seminal vesicle of one animal are introduced by means of the penis into the atrium of its mate. There may be a mutual exchange, but certain observations lead to the belief that the younger specimens in which the male organs are at the height of their development fertilize the older ones having the functional female apparatus, while older ones do not fertilize the younger.

In this regard it is to be noted that during early life the animals have widely distended seminal vesicles; in the old worms this organ contains only small masses of spermatozoa. Moreover the younger specimens in which the ovaries are not functional have not been observed to contain sperm in the atrium and antrum.

Embryos

In a worm which is rapidly reproducing one finds distributed through the parenchyma capsules (fig. 1) containing embryos in various stages of development, from the newly-fertilized egg to ciliated young, actively moving about in their cramped quarters. During early development the two embryos of each capsule are usually separated by a considerable thickness of yolk, but may in some cases lie so close together that the blastomeres of one touch those of its mate. The capsular membrane, heavy and well defined while the embryos are small, becomes gradually thinner on account of water absorption and consequent stretching until, by the time the young develop cilia, it is extremely delicate and apt to be ruptured by vigorous pressure of the surrounding organs. When fully enough developed the young break forth from their capsules, swim freely about in the mesenchyme of the mother for a time, and ultimately, as her body undergoes degeneration, slip through the body-wall to the outside. Frequently one is found in the mother's intestine.

IV. DEVELOPMENT

1. *Growth of the eggs and consideration of the yolk-nucleus (mitochondrial mass)*

Throughout the entire length of the ovary (text fig. 2) from the point where the oogonia are transformed into oocytes there is a gradual increase in the size of the latter. The nucelus measures at the beginning 0.01 mm.; at the time it leaves the ovary its diameter is about 0.025 mm. During all this growth period the nucleus is in a resting condition, showing a fine chromatin reticulum and prominent nucleolus. The latter is at first apparently a sphere whose periphery retains more stain than the center. Often one side of the nucleolus is much darker than the other, so that the pale interior is eccentric. When the cell has passed half the length of the ovary a dark spherule with a light interior appears in the center of the nucleolus (figs. 7, 8), and remains until the cell leaves the ovary.

The term 'resting nucleus' is to be used here only in so far as it applies to the nucleus as a whole; i.e., it retains its spherical or oval form and its limiting membrane. There is observable, however, a certain activity of the chromatin. While the newly formed oocyte nucleus contains a heavy chromatin reticulum whose strands are quite uniform in thickness, the latter soon became markedly thinner. This is due to the accumulation of a part of the chromatin in one to four masses usually near the nuclear membrane (text fig. 3). These are at first irregular, but soon become rounded so as to resemble the nuleolus in form. Rarely do they attain the size of the latter, however, and never contain a lighter interior; an intense blue-black iron-haematoxylin stain is characteristic of these fragments.

It is first at this stage that one often finds one or more black bodies of perfectly spherical outline lying in the cytoplasm outside the nucleus. Their appearance is such as to suggest their identification with the chromatin bodies just described as arising in the chromatin reticulum. If now the nuclei of the cells in whose cytoplasm these black spheres lie be examined, it is found either that they contain only a nucleolus and a fine

chromatin reticulum, or that fewer black masses are present than in the oocytes which are slightly younger. It seems probable then that the oocyte nuclei eliminate a part of their chromatin in the form of spherical fragments which come to lie in the cytoplasm.

Of considerable interest in relation to the nourishment of the embryo is a body always found present in the oocyte after it has commenced its growth. This is the 'Dotterkern' or 'yolk-nucleus.' A light brown color in iron-haematoxylin-eosin
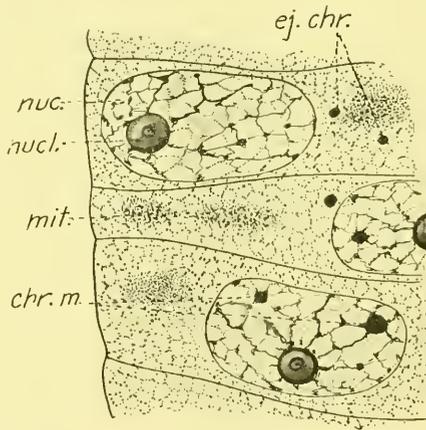
Text fig. 3   Portion of a frontal section of the ovary to show masses of chromatin before and after ejection from the nucleus. *chr.m.*, chromatin mass; *ej.chr.*, ejected chromatin; *nuc.*, nucleus; *nucl.*, nucleolus; *mit.*, mitochondrial mass. × 1016.

preparations brings the yolk-nucleus into contrast with the cytoplasm in which it is imbedded (figs. 4, 6, 7). At a magnification of two hundred fifty its appearance is that of a homogeneous gelatinous mass, sometimes existing as a single compact body, but often as flocculent material in one or more smaller masses of different sizes. The substance consists ultimately (fig. 7) of a multitude of granules which are so extremely fine and so closely grouped that a high magnification is necessary in order to distinguish them as such.

Since the term 'yolk-nucleus' has been applied by different authors to several different cell structures in many groups of animals, it seems advisable to pause here, and briefly to describe these various types. The name 'Dotterkern' was first given by Carus in 1850 to a body which had been described by von Wittich as lying in the cytoplasm of immature spider eggs (Lycosa, Tegenaria and Thomisus). Since then observers have reported the presence of a yolk-nucleus in the eggs of echinoderms, molluscs, worms (Trematodes), crustaceans, myriapods, insects, fishes, amphibians, birds and mammals, including man.

As explained in Korschelt and Heider's Textbook of Invertebrate Embryology, the bodies described for the various animals as yolk-nuclei may be classified in three groups, (a) true 'Dotterkerne' which are supposedly concerned with the deposition of yolk in the egg, (b) bodies more or less similar to yolk-nuclei in appearance, and (c) structures which are to be identified as attraction spheres.

Calkins in his preliminary notice published in 1895 on the origin and changes of the yolk-nucleus in the eggs of the earthworm, Lumbricus terrestris, points out that "the work of various observers may be divided into two classes according as the yolk-nucleus is conceived to be of cytoplasmic or nuclear origin."

As examples of the first class Calkins cited, among others, the work of Lubbock ('61) who regarded the yolk-nucleus as a thickening of the plasm forming the vitellus, of Sabatier('83) who asserted that in the spider the yolk-nucleus arises in the cytoplasm near the nucleus and wanders to the periphery where it degenerates; of Hall ('90) whose observations on the chick led him to the conclusion that the yolk-nucleus has its origin in a mass of granules near the germinal vesicle to which it may send out prolongations; and the work of Monticelli ('92) who claimed that in the Trematodes there is no connection between germinal vesicle and yolk -nucleus, but that the latter arises as a protoplasmic differentiation of the cytoplasm. Jordan ('93) came to a similar conclusion in regard to the yolk-nucleus of the newt.

Of investigators who considered the yolk-nucleus to be derived from the nucleus Calkins mentioned Shäfer ('83) who observed in the fowl's egg that the yolk-nucleus was connected with the germinal vesicle by numerous fine striations; Balbiani ('83) who claimed that in the Geophilidae it originates by direct transformation of the parts of the nucleolus of the germinal vesicle. Leydig ('88) saw the nucleolus in Triton become amoeboid and wander out into the cytoplasm where it finally broke up into granules around the periphery of the yolk. Finally he cites Henneguy ('93) and Balbiani ('93)who both found that in various eggs the yolk-nucleus originates from the nucleolus.

As a result of Calkins' own observations ('95) on the eggs of Lumbricus he concluded that the yolk-nucleus is of nuclear origin. He found it lying on the margin of the nucleus in direct connection at several points with the chromatin; the nucleolus takes no part in its formation. After migrating into the cytoplasm he conceived the granules of which it is composed as separating and forming the yolk plates after undergoing a change in chemical composition.

Wilson ('96), in "The Cell in Development and Heredity" speaks broadly of the yolk-nucleus, conveying the impression that already the body was beginning to be looked upon as not having, perhaps, so much importance as a functional cell organ as had previously been attributed to it. He says in part:

Calkins' observations taken in connection with those of Balbiani, Van Bambeke, and other earlier workers give, however, strong evidence, as I believe, that the 'yolk-nucleus' of Lumbricus is derived, if not from the nucleus, at any rate from a substance nearly related with chromatin, which is afterward converted into cytoplasmic substance. It is certain, in this case, that the appearance of the yolk-nucleus is coincident with a rapid growth of the cytoplasm; but we cannot suppose that the latter grows entirely at the expense of the yolk-nucleus. More probably the yolk-nucleus supplies certain materials necessary to constructive metabolism, and it is not impossible that these may be ferments. We may perhaps interpret in the same manner the elimination of separate nuclear elements, (i.e., not forming a definite yolk-nucleus) as described by Van Bambeke, Mertens, v. Erlanger and many earlier writers.

The meaning of the yolk-nuclei of purely cytoplasmic origin is very obscure, and we have at present really no ground for assigning to them any particular function. It can only be said that their appearance coincides in time approximately with the period of greater constructive activity in the cytoplasm but there is no evidence of their direct participation in the yolk-formation, and we do not know whether they are active constructive physiological centers, or merely stores of reserve substances or degeneration products.

Crampton in 1899 published an account of the 'yolk-matrix' of an Ascidian egg (Molgula). He first noted its appearance in the living primary oocyte as "one or more small masses of highly refractive granules" lying in the cytoplasm at one side of the nucleus. Later, the granules become more numerous and are closely packed together into a cap-shaped mass which partly encloses the nucleus. Various chemical tests led him to the belief "that the yolk-matrix is an albuminous body of the cytoplasm, but perfectly agrees with the albuminous constituents of the nucleus," and that on this ground the yolk-nucleus is either directly derived from the nucleus or is formed under its immediate influence.

After a time the granules constituting the yolk-matrix separate, according to Crampton's observation, and spread throughout the cytoplasm in whose interalveolar substance they came to lie. Finally the granules enlarge to form the deutoplasm-spheres.

In the light of more recent research it would appear that these yolk-nucleus granules are mitochondria, and that Crampton's second alternative as to their origin is the correct one i.e., that they are formed under the influence of the nucleus, rather than directly from nuclear material.

One of the most prominent types of yolk-nucleus was described in 1898 by van Bambeke for the oocyte of the spider, Pholcus phalangioides. First appearing as a small group of granules near the nucleus, it develops into a definitely outlined, more or less cresentic mass lying in a clear portion of the cytoplasm. Increasing immensely in size the body grows around the nucleus, sometimes adhering to it for a time, but finally reaching the height of its development in the form of a cup within whose

hollow the greater part of the cell body lies. In other words, the yolk-nucleus is peripheral. In some sections it appears as a crescent reaching more than half way round the cell; in other planes which are at nearly right angles to the last a ring-like band is the picture presented by the yolk-nucleus.

From this point on Van Bambeke noted a disintegration of the 'corps-vitellin.' The essential difference in the fate of the granules into which this body fragments from that ascribed to them by earlier investigators of other forms, lies in their transformation into fat globules which are subsequently 're-sorbés etassimilés par le protoplasme ovulaire." In view of this van Bambeke suggests that it is on account of the resorption that the protoplasm is able to elaborate the deutoplasmic elements. It will be remembered that Calkins and Crampton recognized a direct transformation of yolk-nucleus granules into yolk spheres.

According to von Adelung in the Zoologisches Centralblat Samassa and Groben described for the parthenogenetic egg of the Daphnid, Moina rectirostris, an intensely staining body of characteristic form, which they called the polar body. Weissman and Ishkawa, however, observed the formation in the living egg of the true polar body. Finally Lepechkine in 1900 determined that the structure observed by Samassa and Groben is a true yolk-nucleus, and characterized it as follows

Er ist dem Ei von dessen Ablage an bis zum 32-zelligen Stadium eigentümlich, wird durch Kernfärbemittel tingiert, gehört von Anfang an einem bestimmten, am animalen Pol gelegenen Blastomer an, ist von bestimmter Gestalt, gleichmässiger Konsistenz und enthält Vakuolen, löst sich beim Zerfall gleichsam in feinste Körnchen auf, wobei diese, dem Kern sich nähernd, denselben umgeben; der Dotterkern steht in Verbindung mit dem Kerncentrosom, welches seinen Zerfall herbeiführt; nach dem Zerfall des Dotterkerns treten in dem entsprechenden Blastomer Fettröpfchen auf, welche auf lange hinaus einem Bestandteil dieses Blastomers sowie seiner Tochterzellen ausmachen; aus dem den Dotterkern enthaltenden Blastomer enstehen die Genitalzellen des Keims; endlich giebt der Dotterkern in den ersten Furchungsstadien ein Plasmaklümpchen ab, welches zwischen die Eioberfläche und die Dottermembran zu liegen kommt.

Was die Bedeutung des Dotterkerns betrifft, so ist der Verf. (mit van Bambeke, Wilson und Häcker) der Ansicht, dass derselbe eine

Rolle im Stoffwechsel spielt, und die Entwickelung und das Wachstum entweder das ganzen Eis oder eines bestimmten Blastomers begünstig.

From the foregoing review it is evident, first, that several different cell structures have been designated as yolk-nuclei, and second, that diverse views have been held as to the origin, function, if any, and the fate of the 'Dotterkern.' In the majority of instances where the latter was traced its disappearance as a concrete cell organ occurred in the oocyte. A part of the yolk-nucleus observed by Balbiani in Tegenaria, however, retained its individuality throughout cleavage and finally became located among the yolk-spheres in the abdomen of the young spider.

Since Lepechkine's work of 1900 a vast amount of literature has appeared dealing with mitochondria. It is apparently to this class of elements that the true yolk-nuclei described by him and earlier workers belong. Therefore it seems justifiable to replace the name yolk-nucleus with one which is more descriptive of its true nature. We propose to use in this paper the term 'mitochondrial mass.'

The cytoplasm or ooplasm of the oogonia is, in well preserved preparations, so finely granular that it resembles an opaque fluid. With the iron-haematoxylin method a light bluish-gray color is characteristic. If the sections be washed too long in the iron alum after their removal from the stain, and subsequently counter stained with orange G, the latter imparts to the cytoplasm of the oogonia and oocytes a yellowish brown tint. In such mounts the mitochondrial mass, which also takes the orange stain, is detected with difficulty. Those preparations, however, which retain a sufficient amount of the blue haematoxylin show the mass even in its earliest stages. Figure 6 was drawn from such an ovary. A small group of brownish granules in the third oocyte which shows a membrane on the right hand side of the ovary is the first indication of the mitochondrial mass. A study of the nuclei in the younger cells does not lead to the conclusion that the granules are of nuclear origin. They are rather to be regarded as bodies which have arisen in the cytoplasm under the influence of nuclear energy.

The question as to whether any function is to be assigned to the mitochondrial mass in Paravortex can be more intelligently considered after the conditions under which it exists as long as it remains visible have been fully described.

At a time approximately coincident with the appearance of the mitochondrial mass the cytoplasm of the oocyte becomes less dense, while its general color changes from the bluish gray of the oogonium to a more neutral tint bordering on the brown. So far as can be observed with the highest magnification obtainable this change in color is due not to an intercalation of yellowish particles but to a change in the staining reaction of the cytoplasm as a whole. The simultaneous increase in the amount of the latter is not caused by deposition of distinct deutoplasm spheres. At any rate there is no addition of particles which stain differently from the cytoplasm. Neither is growth the result of vacuolization; the mature oocytes in well preserved material never contain vacuoles. The only change observable, in so far as the consistency of the cytoplasm is concerned, is a tendency of the minute granules to become grouped in slightly larger units of irregular form. The appearance presented to the eye is that of a multitude of small flakes and flocculent bars between which intervene irregular colorless regions which in size closely approach that of the stained particles (fig. 7). These spaces become somewhat more conspicuous as the oocyte reaches maturity. It has been impossible to detect in them the 'mitosomes' which Wilson and others have described as lying in the protoplasmic trabeculae between the alveoli of the echinoderm egg.

Although, as pointed out above, no conspicuous spheres are to be found in the cytoplasm of Paravortex, it is apparent that the particles which are added must be of the nature of deutoplasm. Their distribution is remarkably homogeneous; in other words, the eggs are homolecithal or alecithal.

Nourishment enters the ovarian cells by osmosis from the parenchyma surrounding them, not, as Patterson states, in the form of particles ingested from the vitellaria which at some points come into close proximity with the ovary. The vitelline

cells contain flakes and globules of a substance which, by the haematoxylin-eosin method, are stained an orange brown, and are in size and density much more conspicuous than the particles suspended in the cytoplasm of the oocyte.

During the growth of the latter the mitochondrial mass has assumed conspicuous proportions. This relative increase in bulk is well illustrated in figure 6, which indicates as well that the substance may be accumulated in a single mass, or irregularly distributed throughout the cytoplasm. One might easily imagine, on account of the uneven edges of the masses from which lines of granules are often seen extending out into the cytoplasm, that the mitochondria are being directly transformed into the deutoplasm particles. Van Bamkebe ('98) points out that it is well to guard against such a conclusion, since he finds in the oocytes of Pholcus that the 'yolk-nucleus' granules first become transformed to fatty drops which are reabsorbed by the protoplasm, and suggests that possibly the latter then manufactures from this nutritive material the true deutoplasm globules. For the present, judgment in this matter of the function of the mitochondrial mass is suspended. It is to be noted that the body increases consistently in bulk while the cell is still in the ovary.

At the time of encapsulation the oocyte has attained a size about four times that which characterized its earliest appearance. The average measurements are approximately 0.112 mm. in width and 0.023 mm. in length, the cells being strongly flattened perpendicularly to the long axis of the ovary.

2. Formation of the capsule and origin of the twins

As to the manner in which the older eggs are nourished, Patterson asserts, "that the ova are at their upper margins absorbing yolk from the glands" (the vitellaria). This altogether erroneous interpretation will be considered below with another concerning the origin of the twins. Again Patterson states that,

In consequence of this rapid growth certain retrogressive changes involving the cell membranes separating contiguous ova frequently

make their appearance. As a result two or even more nuclei may come to lie within one common yolk mass, which occupies the extreme tip of the ovary. In other words, a syncytium is formed here. In the vast majority of cases only two ova are involved so that the usual picture displayed in this region represents a binuclated yolk mass.

Patterson was evidently misled, both as to the relation of the ova to the cells of the vitellaria, and their relation to each other, by the phenomenon of oblique sections across thin membranes. Hardly a better example of this condition could be found than a longitudinal section of the Paravortex ovary, particularly of its posterior end where it turns inward to meet the oviduct. At this angle several of the ova are sure to be cut with varying degrees of obliquity, rendering the egg membranes correspondingly indistinct. The membrane between the last two ova included is the most likely to be cut at the sharpest angle, and hence the most difficult to observe.

Since all the eggs in the ovary are flattened nearly perpendicularly to the horizontal plane, a frontal section of the organ is all that is necessary to prove that very distinct membranes do exist between all ova, particularly at the posterior end of the ovary (fig. 8). A vertical section cut obliquely through the posterior angle of the ovary brings out the same fact.

Patterson's figure, introduced as evidence in favor of his syncytium theory, indicates very clearly to us that the ovary curved inward at the right. The egg having the larger nucleus was pressed into its neighbor containing the smaller nucleus. Since the plane of the section was more tangential to the first than to the second the former appears to be contained within the boundaries of the latter. The membrane between the two appears indistinct, not because it is undergoing degeneration, as Patterson would interpret the condition, but on account of the obliquity of the plane at which it is cut. Many of my preparations present an identical appearance.

In a similar manner I would account for the faintness of the boundaries between ova and vitelline cells in the same figure. Sections through the posterior end of the ovary and the overlying vitellaria are not usually perpendicular at all points to

the membranes of the eggs and of the vitelline cells, owing to irregularities in the contour of the ovary. Wherever the section is not thus perpendicular it is oblique, and hence the membrane is inconspicuous.

The masses of granules which Patterson interprets as streams of yolk entering the ova can be found in eggs which are not in contact with the vitellaria. In my own preparations I have considered these darker lines of granules as portions of the cytoplasm which retained more stain than that the rest of the cell.

It is evident, then, that Patterson was misled, both in regard to the relation of the contiguous ova in the posterior end of the ovary, and the relation of the ova to the vitellaria. Therefore I cannot agree with him, either that the vitelline material is pumped directly into the ova, nor that the breaking down of a membrane between two ova is part of a process by which two or more nuclei are enclosed in one capsule. No syncytium is found here. I am not content with the qualification which Patterson introduces as follows:

"However not in all cases do the two contiguous ova lose their intervening membranes, but some become completely surrounded by vitelline cells, which through a process of disintegration, form the yolk mass of the definitive capsule." The egg membranes are never so lost. It can be shown that in order reasonably to account for the multiplicity of embryos in capsules of Paravortex gemellipara it is unnecessary to resort to such an extraordinary mechanism as a syncytium in the ovary.

As to the manner in which two embryos come to lie in one capsule two possible hypotheses remain; first, that polyembryony occurs in P. gemellipara, or second, that two or more ova are liberated, and, together with yolk cells from the vitellaria, become simultaneously enclosed within one capsular membrane. That the first hypothesis is false there is no room for doubt. Capsules have often been observed in which both ova are undergoing maturation, a state which precludes the possibility that a single fertilized egg was enclosed in a capsule and

subsequently divided into two cells, each capsule developing into an embryo.

There remains the hypothesis that any capsule which contains a certain number of embryos originally had deposited in it the same number of eggs. A priori it is to be expected that such a process occurs here, for both von Jhering ('80) and von Böhmig ('86) observed in the uteri of the oviparous parasites Graffilla tethydicola and G. muricicola respectively capsules containing usually two ova; Böhmig states that each ovary contributes one egg. The same mechanism is probably involved in the formation of the capsules of the oviparous specimens mentioned above and in the viviparous Paravortex gemillipara. In the former two species the capsules pass out of the animal through the atrium and genital pore, while in the case of P. gemellipara they never enter the atrium, but break out of the antrum into the mesenchyme.

Finally Hallez ('09) observed the formation of the capsule in Paravortex. To quote:

Chez Paravortex cardii, comme dans l'espèce précédente, il n'existe ni utérus, ni cavité d'aucune sorte au point de jonction du lécithogène, de l'ovaire et de l'oviducte.

Quand le cocon commence à se former, il se produit un afflux de cellules vitelline autour des ovules mûrs qui ne sont pas encore complètement détachés de l'ovaire, afflux qui distend la membrane propre de l'ovaire et refoule le tissue conjonctif latéralment, c'est-à-dire du côté où la résistance est moindre. Il se forme ainsi une hernie qui s'accroît davantage à mesure que le nombre des cellules lécithofères augmente autour des ovules mûrs . . . .

On observe à ce moment des spermatozoïdes dans la partie de l'oviducte voisine de l'ovaire . . . , tandisque l'atrium femelle et une partie de l'oviducte contient un cylindre d'une substance amorphe, transparente, coagulée par les réactifs . . . . Cette substance, qui ne peut être que la produit de sécrétion des glandes coquillières, adhère par places à la paroi de l'atrium comme si elle était glutineuse.

Hallez finds that from two to four ova are thus inclosed in a capsule. Since the ovaries of P. cardii consist at their posterior ends of several eggs, there is greater likelihood that more than one at a time will be freed than there is in the case of P. gemellipara. As a matter of fact Hallez finds a larger number

of capsules containing three or four embryos than is met with
in the latter species.

a. *The process in Paravortex gemellipara.* The general plan
and appearance of the ovaries, vitellaria and antrum of P.
gemellipara supports the hypothesis that a similar process occurs
here. As described in the introduction, the antrum femininum
bifurcates at its distal end, sending a thin-walled branch, the
oviduct, to meet the ends of the ovaries and vitellaria of each
side.

Examination of the posterior end of either ovary in animals
which have reached the egg-producing stage usually shows that
the last, and hence the ripest, egg is being pressed inward to-
ward the antrum by the ova behind it. Posteriorly the com-
pact vitelline cells crowd the egg in a forward direction so that
it appears, in a frontal section, as in figure 4. Here the ter-
minal egg of each ovary is being subjected to the same conditions.
The one on the right seems just in the act of being forced into
the oviduct. Such is the usual picture, an egg from each ovary
on the verge of entering the oviduct. Only a slightly greater
pressure is required to complete the process. Suppose the
worm were now to contract its body in this general region; the
probable result would be that both ova would enter the antrum.

Most conclusive visual evidence is to be gained from a series
of sections of a specimen which had been killed in the most criti-
cal stage, where two eggs had just been set free and were being
surrounded with the yolk cells from the vitellaria. The sections
are slightly oblique between a vertical longitudinal and a frontal
plane, somewhat nearer the latter.

Figure 9 drawn from the specimen under consideration,
shows what is taking place. Apparently the right ovary, part
of which is included in the section, was distorted and crowded
inward by the contraction of the animal in the fixing fluid.
Otherwise it would have been impossible to have cut a section,
which would include, as this does, seminal vesicle, antrum and
ovary. Aside from the retention of a deeper stain these two
ova are in the same condition as the eggs at the end of the ovary.
The nuclei of both are resting, and their chromatin and nucleoli

are typical of ripe ova in which maturation has not yet begun. Owing to a relief of pressure both eggs are more nearly spherical than those in the ovary.

The yolk cells are arranged about these eggs in a loose envelope on all sides except that toward the ovary. Between the yolk cells and the eggs numerous spermatozoa appear. The latter can be traced throughout the length of the antrum and into the atrium where they form a compact mass.

This specimen leaves no doubt that two normal ova have been freed into a temporary cavity formed at the thin-walled distal end of the oviduct and partly bounded by the ovary, vitellarium and mesenchyme, and are being surrounded with a number of vitelline cells, between which numerous spermatozoa were actively moving at the moment of fixation. No proof is at hand that one of the eggs came from the right ovary and the other from the left, nor that both came from the right; either case is possible. Their presence near the end of one ovary may signify that both ova were derived from the latter. On the other hand, the possibility is not excluded that one egg came from the other ovary, and by pressure of the tissues was quickly shifted to its present position. In favor of this view it is to be observed in this series of sections that, while the right hand ovum is still in contact with the outer corner of the last egg in the ovary, its mate is entirely free, and has assumed a more nearly spherical shape—two characteristics which an egg would probably possess had it come from the other side of the body.

That one ovary may contribute two ova to the formation of some capsules, is clear when it is known that occasionally three embryos are found in a capsule. As stated in the introduction, two is the normal number, but Linton ('10) and Patterson ('12) both observed capsules with one and three embryos. My material shows further examples of both; and in addition one capsule containing four. Thus it is possible that the two eggs deposited in any capsule may have had a common source, or may have come from opposite sides of the body. The weight of the evidence favors the latter as being of most common occurrence in Paravortex gemellipara.

In figure 9 are shown several masses of a homogeneous substance which is altogether unlike anything observed in other preparations. This latter fact, together with the arrangement of the masses—more apparent in other sections of the same capsule—in a half circle about the yolk cells which surround the two ova, suggests that the substance is the fluid secretion from the shell glands in the process of being laid down in the formation of the capsular wall. A similar condition was observed by Hallez ('09) for Paravortex cardii.

As the above quotation from his paper points out, Hallez asserts that there is no uterus in Paravortex cardii nor a cavity of any sort at the point of junction between vitellarium, ovary and oviduct. Still he figures a direct continuation of the lumen of the oviduct into the cavity which is receiving a mass of yolk cells. It is true that P. gemellipara possesses no permanent, distinctly limited uterus by whose contractions the yolk cells and ova are molded into a spherical mass to which by accretion the capsular fluid is applied. Nevertheless it is apparent that in P. cardii and P. gemellipara the process is essentially the same as in oviparous worms, differing only because the encapsulation cavity is bounded partly by the ruptured and distended end of the antrum femininum and partly by the vitellaria, mesenchyme and ovary. The cavity is indefinite and probably never exactly the same for successive capsules, but there is, in spite of the delicateness of the tissues, sufficient resistance to round off the capsular contents and allow of the laying down of the shell. Thus the process is essentially in agreement with that found by other investigators in related species.

b. Description of the newly formed capsule. The appearance of a capsule which has just passed from the reproductive organs into the body parenchyma is clearly shown in figures 10 and 11. It lay close outside the uterus, slightly posterior and ventral to the point at which the ovary turned inward to join the vitellaria. It should first be made clear that, whereas living capsules due to their turgidity are smooth in outline, preserved material shows capsules somewhat shrunken and crowded out of shape by neighboring tissues.

The shell membrane is clearly visible, on one side closely opposed to the yolk, while on the other a shrinkage space intervenes. This capsule contained but one egg. That illustrated in text figure 4 shows the two ova at once. Only exceptionally do eggs lie so near the surface as indicated in figures 10 and 11; usually they are until late cleavage entirely surrounded by yolk. In figure 11 the plane passed through portions of eleven yolk cells, of which there were, as determined by a count of the visible nuclei, thirty-six originally enclosed in the capsule. Hallez found from ninety to one hundred eighty-seven in various capsules of P. cardii. As may be seen by referring to figure 10 of another section through the same capsule several cells have already lost their membranes; only the degenerating nuclei remain to indicate the number of cells.

   c. Behavior of the vitelline cells. Up to this point, with the exception of the yolk nucleus above described, the structure and behavior of the reproductive organs and their products are remarkably similar in P. cardii and P. gemellipara. From here on, however, the latter presents characteristic differences as to the fate of the vitellarial yolk-cell nuclei, absorption of the yolk and the formation of the ectoderm.

   The ectolecithal yolk cells in the P. cardii capsule, according to Hallez, soon lose their membranes, those near the center retaining them the longest. About one-half of their nuclei, it is to be noted, degenerate, while the remainder come to lie in the portion of the yolk which stains like cytoplasm. From this portion the 'ergatoplasmic' granules of yolk have previously separated and collected in 'balles vitellines.' This second form of yolk stains distinctly with eosin. The cytoplasmic-like yolk occupies the periphery of the capsule, sending a prominent lamella inward between the embryos—now in process of segmentation—and secondary "parois alvéolaires plus où moins complètes autour des masses éosinophiles." With its 'migratory' nuclei the cytoplasmic yolk stands as a syncytium surrounding embryos and 'eosinophile' yolk. Hallez is of the opinion that these migatory nuclei are effective in changing the nature of the

yolk in the 'balles vitellines.' To carry out this function they migrate inward from the periphery.

He finds that the cytoplasm surrounding one of these migratory nuclei makes its way into the substance of each vitelline sphere, eventually drawing with it the nucleus. Later many of these nuclei again emigrate and may then enter into the formation of the ectoderm or of the primary intestine.

Thus it is to be borne in mind that, if Hallez's interpretation be correct, both the entoderm and at least a part of the ectoderm cells in the definitive embryo contain nuclei and cytoplasm which have undergone little change since leaving the vitellaria; in other words, the relationship of these cells to those derived from the egg is apparent only when one recalls that ovary and vitellarium were at their beginning differentiated from the same mass of cells.

Bresslau ('04) traced the history of the yolk cells in Mesostomum ehrenbergi, M. productum, M. lingua, Bothromesostomum personatum and Plagiostomum girardi. The yolk cells of the former behave like those of P. cardii in that they soon separate into two groups. The outer ones become flattened so as to form a shell membrane between the capsular shell and the inner yolk cells. The latter become vacuolated to such a remarkable extent that the nucleus and a small amount of cytoplasm is finally flattened in the form of a thin cresent on one side of the vacuole As the ectoderm formed from the anterior portion of the developing embryo envelops these yolk cells, the nuclei of the latter gradually degenerate, but may still be discerned between the larger vacuoles after the ectoderm has closed over the posterior end of the embryo.

No such shell membrane is formed in the capsule of Bothromesostomum personatum; here the much more rapid degeneration of yolk nuclei is accompanied by the appearance of multitudes of vacuoles of various sizes, all much smaller than those described for M. ehrenbergi. The formation of the ectoderm and enclosure of the yolk globules within the embryo is essentially the same as in the latter species.

The development of M. lingua and M. productum is so similar that Bresslau figures only the former. While a limited number of yolk-cell nuclei come to lie next to the shell the others rapidly disintegrate. No distinct shell membrane is indicated

The newly formed capsules of the Alloiocoele, Plagiostomum girardi, are stated by Bresslau to contain from ten to twelve eggs distributed amongst several hundred yolk cells. The latter then arrange themselves in groups about the several eggs. In the behavior of the yolk-cell contents a notable difference is to be observed in comparing it with the process of yolk transformation in Mesosotomum. The vitelline substance consists of a large number of small refractive spherules which collect at the periphery of the cells. Gradually the cell membranes disintegrate, the process setting in first next to the egg. Since the nuclei, like those in M. ehrenbergi, remain distinct for some time a yolk syncytium results. Vacuolization does not occur until after the embryonic ectoderm has begun to grow out over the yolk. As in three of the four species of Mesostomum a small number of yolk-nuclei become flattened on the surface of the vitelline mass, while the remainder, although less conspicuous than at first, remain visible, scattered through its interior until the ectoderm has enveloped the yolk. Here again no shell membrane is developed form the yolk cells.

For the Dendrocoeles, Planaria torva, P. polychroa and Dendrocoelum lacteum, Mattieson ('04) found that about one hundred and fifty of the yolk cells immediately surrounding the egg become separated from those farther removed and pass over into a syncytium. This change takes place before the enclosed embryo has reached the two-celled stage. Mattieson concluded that, even previously to the formation of the syncytium, an active interchange of material between the egg and yolk cells takes place. The nuclei of the yolk cells which enter this syncytium slowly degenerate, but are still met with at the stage when the pharynx is differentiated.

Two ectoderms are developed in these Dendrocoeles. First a transitory epithelium is formed by blastomeres which migrate through the yolk and arrange themselves in an extremely thin

sheet over the surface of the yolk syncytium. Later the definitive ectoderm, arising also from derivatives of the blastomeres, is laid down beneath the first.

To summarize: Bresslau ('04) observed the development of a distinct shell membrane about the yolk mass in the capsule of Mesostomum ehrenbergi. It was derived by a differentiation of the outermost vitellarial cells. In Mesostomum lingua and Plagiostomum girardi he found occasional nuclei lying in small masses of cytoplasm at the periphery of the yolk spheres, while in the case of Bothromesostomum all of the yolk nuclei degenerated; none showed any tendency to form a membrane at the surface of the vitelline mass. On the other hand Mattieson ('04) determined that a 'primary ectoderm' was established about the yolk-syncytim of Planaria torva, P. polychroa and Dendrocoelum lacteum by the union of migatory blastomeres.

According to Hallez's interpretation, certain of the yolk-cell nuclei in Paravortex cardii not only form a peripheral syncytium outside the embryos and the nutritive portion of the yolk, but later enter into the formation of the permanent ectoderm as well as the primary intestine.

The behavior of the yolk cells enclosed in the capsule of P. gemellipara resembles that in the case of Bothromesostomum personatum in that all their membranes immediately disintegrate. The process is so rapid that in two hundred and seventeen worms sectioned only one capsule (fig. 11) was encounted where a considerable part of these cell membranes remained intact. As in Bothromesostomum also, the yolk-cell nuclei at once begin to degenerate. Ordinarily they have disappeared altogether by the time cleavage is well under way, but occasionally degenerate masses of chromatin embedded in small islands of nucleoplasm remain visible until the primary entoderm cells have been differentiated. No membrane or temporary ectoderm is formed by the peripheral yolk cells.

The yolk in perserved material, whether enclosed within a cell membrane or lying free in the capsule, consists of multitudes of small globules and flakes lying in a clear plasma. On the whole, the appearance of the yolk in a newly-formed capsule

is very similar to that in the vitellaria. Since in living yolk all particles exist as either spherical, oval or oblong granules (figs. 12, 15) it is probable that the flakes present in preserved material are globules exploded by the action of reagents.

The nuclei of the cells shown in figure 11, whose membranes still remain intact, are already beginning to disintegrate. Succeeding stages of degenerating yolk-cell nuclei appear throughout this section as well as that represented in figure 10.

Occasionally individual yolk cells, perhaps endowed with unusual vitality, retain the membrane until late cleavage in the condition presented when the cell was enclosed in the capsule.

3. Maturation and fertilization

a. Insemination. That the egg in P. gemellipara may be entered by the spermatozoon immediately after leaving the ovary is plainly indicated in figure 9. which shows several spermatozoa between the two eggs and surrounding yolk cells. A careful search through all the sections of these ova yields no evidence that either one has been penetrated.

Each egg of another capsule, illustrated by text figure 4, about which the shell membrane had been formed, contains a spermatozoon. Either insemination has just taken place or the spermatozoon remains quiescent for some time before forming the male pronucleus, for both of these show plainly the long, deeply-stained head and a delicate coiled flagellum.

Without doubt many spermatozoa become enclosed in each capsule, where, as Patterson points out, their identity becomes obscured by the yolk granules. Occasionally a mass of sperm occupies a vacuole in the yolk; such a condition is shown in text figure 5. Presumably the sperm cells continue active in the capsule for some time, so that any egg that is not inseminated at the time it enters the capsule may later encounter the sperm.

Several other eggs have been observed in which the male pronucleus had become lobulated or amoeboid in shape, and had approached, in some a lesser and in others a greater distance toward the female element (text fig. 7).

b. Maturation. Patterson described an achromatic figure as appearing in the egg before it leaves the ovary, and remaining until after the formation of the capsule. In his paper ('12) on Graffilla (Paravortex) gemellipara he gave some space to an account of this spindle, noting the remarkable characteristics of its behavior. Although he believed at that time that the spindle later disappeared, he came to the conclusion after a study of the uterine spindle of Planocera that it was not entirely eliminated, but simply contracted to so small a size as to escape easy detection, and was only an early condition of the maturation spindle.
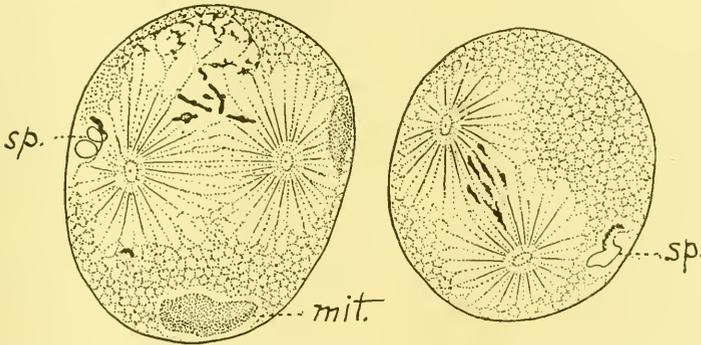
Text fig. 4   Section through two recently fertilized eggs of one capsule.   Yolk and capsular membrane not shown.   *mit.*, mitochondrial mass; *sp.*, spermatozoon.   × 730.

Although occasionally a faint suggestion of a radial arrangement of the granules is perceptible in the yolk-nucleus mentioned above, in all my material no egg shows a true spindle until it has left the ovary and has become surrounded with yolk cells in the distal end of the antrum. It has, of course, for sometime been accessible to spermatozoa. A single egg was observed thus enclosed in loosely massed vitelline cells. It contained a large spindle occupying nearly the entire diameter of the egg. An exact count of the chromosomes in this spindle is impossible on account of poor preservation, but certainly more than the reduced number, four, are present This fact,

with the absence of any polar body, stamps it as the first polar spindle. Its appearance thus before the egg has been enclosed in its capsule is believed to be unusual.

The first maturation spindle typically appears after the two ova and their yolk cells have been encapsulated. At the time of its greatest length the spindle occupies nearly the entire polar
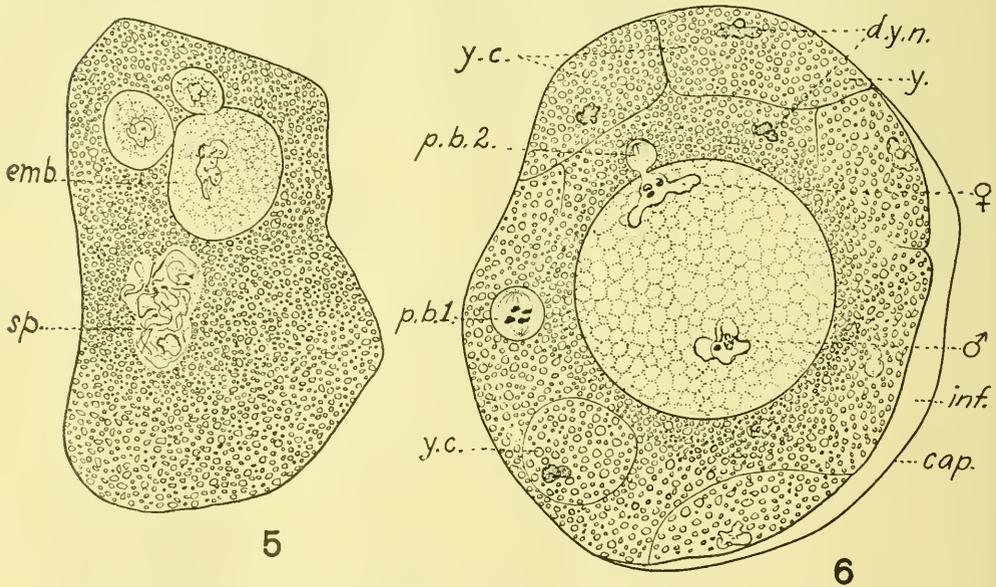
Text fig. 5 Section of a capsule containing in the yolk a vacuole filled with spermatozoa. *emb.*, embryo; *sp.*, spermatozoa. × 400.

Text fig. 6 Reconstructed section in a plane perpendicular to actual sections through a capsule containing an egg which has given off both polar bodies. *cap.*, capsule; *d.y.n.*, degenerating yolk cell nuclei; *inf.*, infiltration; *p.b.1*, *p.b.2*, first and second polar bodies; *y*, yolk; *y.c.*, yolk cells. × 730.

diameter of the egg (text fig. 8) Each centriole appears as a tiny dot in the center of a large centrosphere, from which radiate the conspicuous astral rays. Owing to the minuteness of the chromosomes, and the extreme difficulty in obtaining all the stages of the process of maturation, it is hard to determine in any given case just what phase they represent. One must depend rather on the presence or absence of polar bodies and

spermatozoon, and upon the number and position of the chromosomes, than upon the shape of the latter.

The egg at the left in the capsule in text figure 4 has extruded no polar bodies, contains a spermatozoon and the chromosomes are just being drawn from the disappearing germinal vesicle. Its mate agrees with it in the first two particulars, but shows an advance in that eight small masses of chromatin, which I conclude to be diads, are present in the spindle; in other words, the cell is in the early metaphase. Text figure 8 pictures a similar condition, except that it is impossible to distinguish all the chromosomes. One centrosphere has already divided, while the other is in the process of division.

Hallez ('09) determined that sixteen 'caryomerites' are present in the first polar spindle of the egg of P. cardi. Eight of these are eliminated in the first polar body. The second maturation spindle contains, as he expresses it, "four chromosomes (= eight caryomerites), half of which enter the second polar body."

My material yielded but a single instance where the first polar body had been given off, and the second maturation spindle was forming. In this egg (fig. 9) the first polar body, not quite detached, has already begun to degenerate, as indicated by the scattered chromatin. The centrosome remaining in the egg has divided to form the second polar spindle whose fibers are drawing the four diads toward the equatorial plate. Another section contains the spermatozoon which has begun to enlarge to form the male pronucleus.

In the preparation which best shows the first and second polar bodies the capsule was sectioned in such a plane that portions of the second polar body, though still connected with the egg by a protoplasmic bridge, lie within two of the serial sections, neither of which passes through the egg itself. A third section, however, contains a thin tangential portion of the egg showing the female pronucleus with small masses of chromatin. Text figure 6 represents a reconstruction of this capsule. The female pronucleus is lying just inside the vitelline membrane in the animal pole of the egg. Lobule formation has again set in.
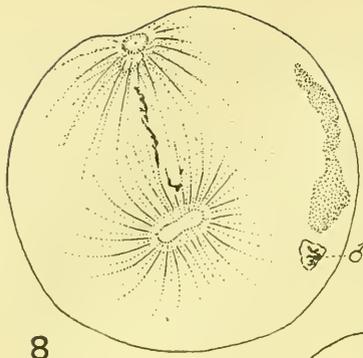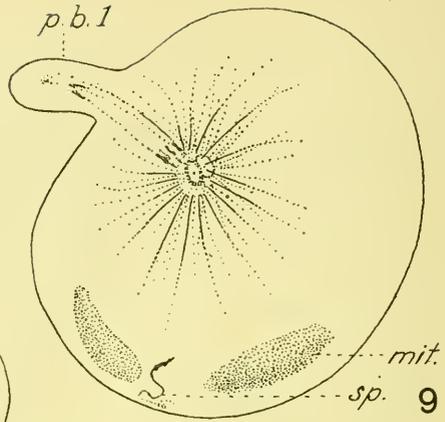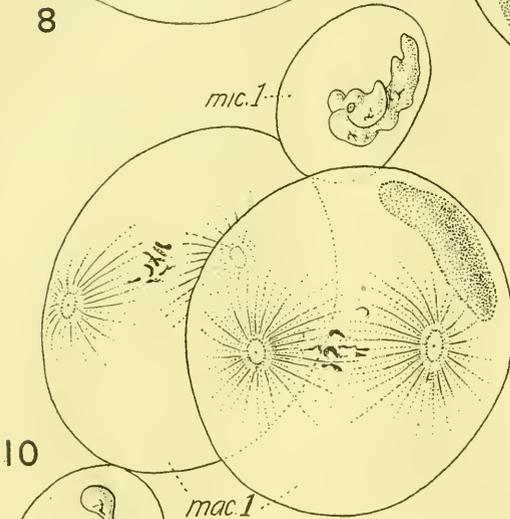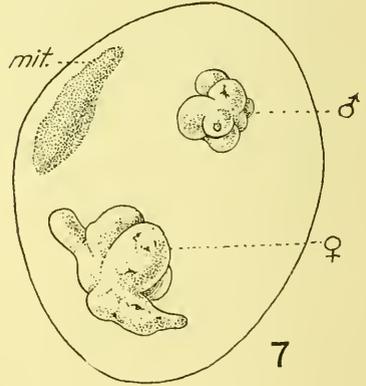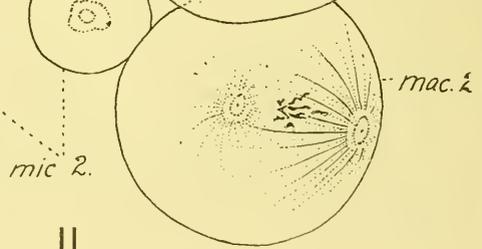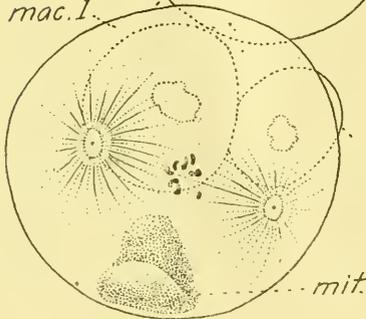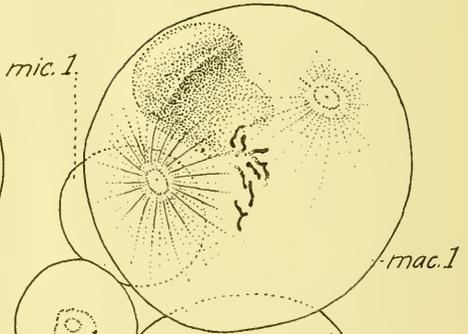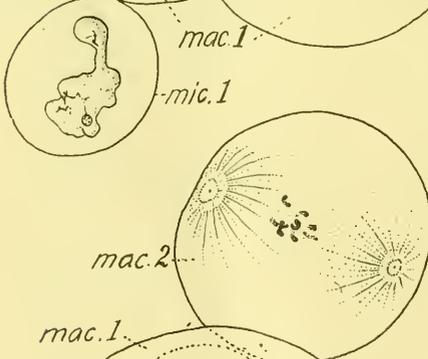
The first polar body at the left of the ovum has approximately twice the diameter of the second. That is it unmistakably smaller than the first two micromeres can be determined by comparing it with the first cleavage figures seen in text figures 10 and 11, while in text figure 12, drawn from life, the polar bodies and first micromere can be directly compared.

c. Fertilization. As stated above, insemination occurs before the onset of maturation. The point of entrance, illustrated by Patterson's figures and by text figures 6 and 9 of this paper, is somewhere in the hemisphere opposed to that from which the polar bodies are extruded. During maturation the sperm nucleus, after losing its flagellum, moves toward the center of the egg, at the same time enlarging to form the irregular male pronucleus.

Before their union both pronuclei become curiously amoeboid or lobular in form (text fig. 7). Mattieson ('04) noted this phenomenon in Planaria torva and P. polychroa, and Bresslau ('04) observed it in several Rhabdocoeles. The same was observed by Hallez ('09) in P. cardii.

The male pronucleus in text figure 6, having entered at some point near the vegetal pole, has penetrated the egg for about one quarter of its diameter. A distinct darkly stained mass of chromatin occupies the center of the otherwise chromatinless vesicle. Since, unfortunately, this preparation was rather heavily stained it is impossible to discern with certainty any degenerating remains of the sperm flagellum among the count-

Text fig. 7   Egg showing the lobulated male and female pronuclei.   × 730.

Text fig. 8   Thick section of an egg containing the first polar spindle. One centrosphere has divided and the other is in the process of division.   ♂ male pronucleus.   × 730.

Text fig. 9   Section of an egg from the same capsule as the preceding. The first polar body is just being given off.   *sp.,* spermatozoon; *mit.,* mitochondrial mass; *p.b.1,* first polar body.   × 730.

Text fig. 10   Reconstruction from sections of a pair of embryos in both of which the first micromere has been given off.   *mac.1.* first macromere; *mic.1.* first micromere.   × 730.

Text fig. 11   A pair of embryos from one capsule, each consisting of two macromeres and two micromeres.   *mac.1, mac.2,* first and second macromeres; *mic.1, mic.2,* first and second micromeres.   × 730.

less dark granules in the egg cytoplasm. It is plainly seen, however, in the earlier stages illustrated in text figures 4 and 9.

I can furnish no evidence concerning the union of the pronuclei; the most careful search failed to disclose any trace of centrosome and aster associated with the male pronucleus.

4. Cleavage

In the study of early cleavage in living capsules a serious difficulty has been encountered in the thick bed of yolk which
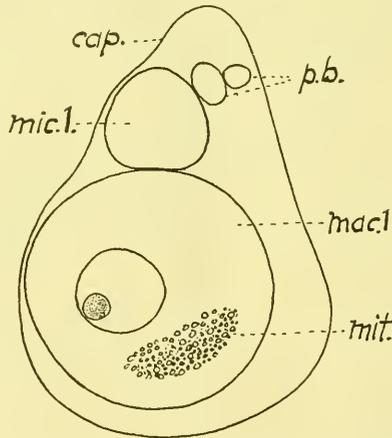
Text fig. 12 Outline from life of a young embryo observed in the antrum of the mother. *cap.*, capsular membrane; *mac.1*, first macromere; *mic.1*, first micromere; *mit.*, mitochondrial mass; *p.b.*, polar bodies. × 730.

surrounds the embryos. Text figure 12 was drawn from an egg, which, contrary to custom, had entered the antrum, become surrounded by the capsular membrane, and, since no yolk cells were enclosed, was conveniently easy to observe. If an attempt be made to remove the embryos at this stage they fragment. Late blastulae may be thus removed with some success (fig. 15).

The only alternative was a trial of various stains. As yet I have not had much success in staining the embryonic cells and at the same time leaving the yolk sufficiently transparent. Neutral red and methyl green were used to stain *in vivo*. Even

a dilute solution of the former acts very quickly on the yolk granules, so that no clear idea of early cleavage was gained by its use. Methyl green, a stain for living nuclei, left the yolk too opaque to allow any satisfactory determination of its effect on the embryo beneath. Acetic carmine and methyl blue, besides killing the embryo, again stain the surrounding yolk too strongly.

In later stages of development, after the embryos have migrated to the surface of the yolk or have taken it into their bodies, all the stains mentioned above can be used to advantage.

It is evident then that we must depend upon sectioned material for observations of the progress of cleavage and gastrulation. More or less uncertainty inevitably follows from the various degrees of contraction which the capsules undergo during fixation. (Hallez was partially successful in avoiding contraction by first placing the worms in sea water containing a little chloral-hydrate or cocaine.) The effect of displacement upon the embryonic cells is, of course, greatest in early cleavage. Earlier investigators have noted that particularly in Turbellarian development there is a very weak affinity between the neighboring blastomeres. Bresslau ('04) shows clearly in Plagiostomum girardi and that the blastomeres normally separate for distances often equal to their diameters; this phenomenon is evident as late as the twenty-four celled stage. Hallez's figures show in Paravortex cardii also a loose group of blastomeres. Later the cells draw together to form at first a rather loose, then a compact mass.

So in the case of P. gemellipara, although the blastomeres lie normally in fairly close apposition to one another, their mutual bonds are easily broken by outside pressure. As a result, sections often reveal isolated blastomeres crowded for some distance into the surrounding yolk.

For the reasons above noted it seems inadvisable to attempt any detailed investigation of cell lineage in this organism. Certain major characters, however, can be consistently observed.

a. *Micromeres and macromeres.* The first cleavage division of the egg of Paravortex gemellipara, as of P. cardii, gives rise

to a pair of cells between which there is a marked disparity
in size. Text figure 10, a reconstruction of the two embryos
of one capsule, shows very well the relative proportion in size
of the first micromere to the macromere. It also indicates that
gravity may not be sufficient to determine from which pole of
the egg the micromere shall arise, for in this capsule the polarity
of one egg is exactly opposed to that of the other. Not too
much reliance, however, is to be placed upon the polarity as
indicated in sectioned worms. When the animals are dropped
into the killing fluid the tissues are as likely as not to become
fixed while the specimens are inverted. Hence if the embryos
rotate with respect to gravity, their positions with respect to the
mother might easily be changed while the fixing fluid was pene-
trating her body-wall and parenchyma. But since both embryos
are equally free to rotate, the evidence afforded by text figure
10 suggests that gravity does not wholly determine the embryo's
polarity. Indeed, this figure, taken in conjunction with figures
14 and 18, indicates that the opposed polarity invariably
exhibited by the two embryos of a capsule in later stages is
already determined at the time of the first cleavage of the egg.

According to Patterson's account the first cleavage of the
fertilized egg of P. gemellipara results in the splitting off of a
micromere of such a size that it is necessary to count the chromo-
somes in order to distinguish it from the first polar body. We
find, however, (text fig. 12), that this micromere is at least
twice as large as the polar body, and furthermore (text fig. 10)
that its nucleus immediately enters a resting stage during which
it may be more or less amoeboid. No such nucleus has been
found in the polar bodies. Owing to the rapidity with which
the latter disintegrate, it is often impossible to find both the
first polar body and micromere present together so as to com-
pare them directly.

It is characteristic of the blastomeres of Paravortex gemelli-
para, as of P. cardii and other Turbellaria, that the nuclei are
strikingly vesiculated.

Hallez asserts that in P. cardii the first micromere divides
before the first cleavage of the macromere. Text figure 10

of this paper would indicate, on the other hand, that, since in both embryos the micromere contains an amoeboid nucleus while the macromere shows a spindle, the latter is about to divide before the first undergoes division. The second cleavage of the large macromere, judging from the conditions represented in text figure 11, results in the formation of two smaller macromeres somewhat unequal in size. The positions of the spindles which are inaugurating a further division of the two macromeres of both embryos indicate that the cleavage is to be of the spiral type described by Bresslau for Mesosotomum ehrenbergi. He found, however, that there were three micromeres present before the second macromere was cut off. Three of the macromere spindles in this pair of embryos are in the prophase, and clearly show eight chromosomes, being drawn into the equatorial plate. On account of an artifact in one section of the fourth macromere the arrangement of the chromosomes could not be determined.

It is important to notice that during early cleavage the mitochondrial mass lies apparently inert at one side of the macromere and entirely outside the spindle. Not until later does the mass itself become divided.

Hallez determined the number of chromosomes present in the equatorial plate of the cleavage spindle of P. cardii to be "four (= 4 groups binaires)." In P. gemellipara, however, the equatorial plate always contains eight U-shaped chromosomes; in the telophase each pole of the spindle likewise holds eight (text figs. 13, 14).

Beyond the four-celled stage cleavage proceeds irregularly as observed by Bresslau, Hallez and others. One can determine, however, that at one pole of the embryo the micromeres become more and more numerous; two macromeres occupy the other pole.

While the macromeres give rise by division to some of the larger micromeres, the latter redivide again and again.

The embryos represented in text figures 13 and 14 contained respectively fifteen and seventeen blastomeres. Text figure 14 is the more normal in that the group of cells is compact with-

out intercellular spaces. The embryo illustrated by text figure
13 had probably been subjected to a considerable degree of
contortion. It is noticeable that the blastomeres in well pre-
served material are closely in contact with yolk, while in shrunken
preparations wide spaces intervene; consequently the blasto-
meres are apt to become displaced. In both figures the pair
of larger cells is prominent. The mitochondrial mass still
remains in one of the macromeres. Text figure 13 shows it
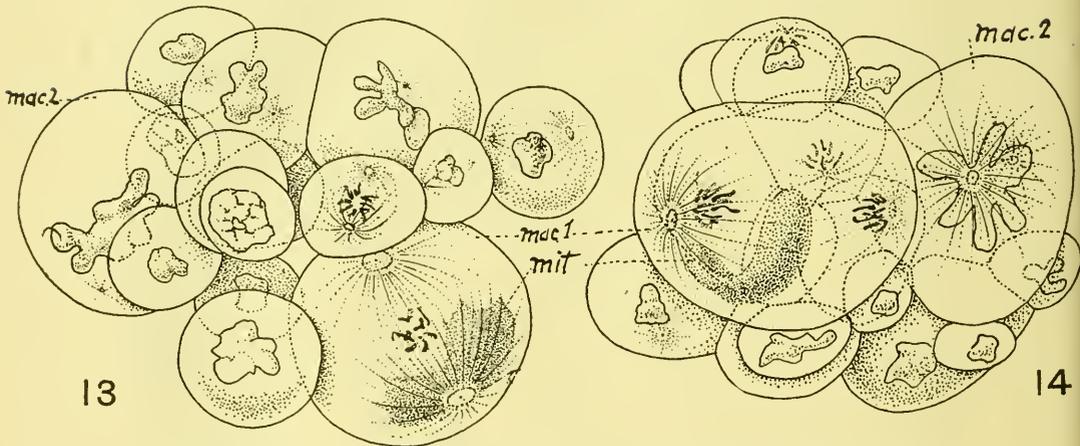
Text fig. 13 Reconstruction from sections of an embryo of fifteen blasto-
meres. *mac.1, mac.2*, first and second macromeres; *mit.*, mitochondrial mass.
× 730.

Text fig. 14 Reconstruction from sections of an embryo of seventeen blas-
tomeres. × 730.

in the form of a hemisphere held at one pole of the spindle, while
in text figure 14 it lies, cresent-shaped, outside the astral fibers.

b. *Behavior of the mitochondrial mass.* Although a careful
search was undertaken, no cleavage figure was found in which
the mitochondrial mass was first being divided. Between the
stages shown in text figure 13 and in figure 20 no intervening
step was discovered. However, the condition at a later stage,
figure 19, suggests that, as the membrane is drawn inward dur-
ing cleavage of the macromere, the mass is too large to remain
wholly in one cell and is so constricted that part is handed

on to each of the daughter cells. It is possible, on the other
hand, that the division is effected directly by the traction of
the spindle fibers upon the mass. Very likely both factors
act simultaneously.

Certain it is that beyond the stage where the mitochondrial-
mass appears in two or more blastomeres the centrosome lies
in its center. Previously the centrosome was difficult to detect
among the numerous granules in the cytoplasm, but now that
it has come to lie in a more finely homogeneous substance, it
is conspicuous. Figures 14 and 16 plainly show it in a clear
region at the center of the mitochondrial mass. Had its his-
tory not been carefully traced the picture here presented would
suggest that the whole structure answered to the centrosphere
containing a centriole. That the black dot represents more
than a mere centriole is indicated by its further behavior. Figure
19 shows that the solid dot becomes enlarged as the new spindle
forms, and contains a light center. Ultimately the body assumes
the form of a sphere whose shell consists of a multitude of tiny
granules, and at whose center lies the true centriole. Further-
more, it lies outside the mitochondrial mass in one cell of figure
20.

c. Blastula. It has been noted that no distinct segmentation
cavity is formed in the blastula. The conditions at this stage
are well shown in figures 14 and 16 which represent sections of
different embryos, and in figure 15, a drawing of a living embryo
which, with a small mass of yolk granules, was freed from its
capsule. The blastomeres lie in rather close contact with each
other, with the exception that a cap of cells which have received
the portions of the original yolk-nucleus tends to become sepa-
rated from the mass of embryonic cells which is to give rise to the
living tissues of the embryo. A solid morula is, then, typical of
P. gemellipara as of Turbellaria in general and many other lower
invertebrates. But for the cells containing the mitochondrial
mass this morula closely resembles that of Hydra and other
Coelenterates. Bresslau figures for Mesosotomum ehrenbergi a
morula somewhat less compact and more irregular in outline.

His figures of the morulae of Bothromesosotomum personatum and Mesostomum lingua are similar, with slight characteristic variations. Although the blastomeres in the segmentation of the egg of Plagiostomum girardi separate remarkably from one another, Bresslau finds them later drawing together to form a compact mass.

The morula of P. gemellipara lacks the marked bilateral symmetry due to a sagittal cleft which, according to Bresslau, characterizes a corresponding stage in the development of Mesostomum ehrenbergi. In P. gemellipara it has been found impossible to distinguish the dorsal from the ventral surface of the morula. The point of view in figure 15 is from almost directly anterior; any attempt to distinguish here between dorsal and ventral is clearly of no avail; the cells are all alike. On the lower left, however, the posterior primary entoderm cells are differentiated by their larger size.

d. Homologues of the germ layers. While it is true that, as Bresslau says of Mesostomum and other Turbellaria, no definite germ layers are formed in these worms, at least rather distinct germ regions are discernible. As the development of the morula progresses there are produced as derivatives of the one macromere about sixteen blastomeres which receive a portion of the mitochondrial mass. They form a cup-like layer, one cell thick, over the surface of the morula which lies toward the center of the yolk-mass, and serve, as will soon become clear, to indicate the polarity of the embryo; this cap of cells is posterior to the rest of the morula, and is to be looked upon as constituting the primary entoderm. Two other regions can be distinguished in the morula at this stage, (fig. 16), first, the mes-ectoderm consisting of smaller cells which are conceived to be direct descendants of the micromeres, and second, a layer of larger cells with less dense cytoplasm lying between the primary entoderm and the mes-ectoderm. These are derivatives of the second macromere, seen in text figures 11 and 13, which lacked the mitochondrial mass. Since they ultimately give rise to the intestine it is appropriate to call this layer of cells the secondary entoderm.

In the slightly older embryos represented in figures 17 and 18 the secondary entoderm cells are still actively dividing, but most of them are larger than the anterior mes-ectoderm cells.

5. Absorption of yolk by the primary entoderm

Toward the end of the morula stage a remarkable process of nutrition is inaugurated. As far as has been determined from the literature no closely similar process has been described as occurring in the nourishing of any embryo. The primary entoderm cells, which were seen in figures 14 and 16 already to have but a loose connection with the main embryonic mass, now apply themselves like amoebae to the surface of the yolk which surrounds the posterior half of the embryo. The firmness of their attachment is evident in figure 17; while the embryos have shrunken away, most of the yolk entoderm cells remain closely in contact with the yolk.

It is the function of these cells to absorb a large part of the yolk mass brought into the capsule inside the vitelline cells. The membranes of the latter have all disintegrated, leaving the yolk flakes and granules free in the capsule. Inspection of the living capsule shows the particles in vigorous Brownian movement. There is, therefore, no obstacle to the action of the entoderm cells. They are shown in figure 17 plainly acquiring the yolk particles. Their nuclei are large, distinct, and according to the rule in active cells throughout this animal, markedly lobulated.

The nature of the yolk granules suffers little change during the process, for those inside the entoderm cells have the same form and color as those lying just outside the cell membrane. It is to be noted that all exist as spherules; no flakes such as constitute a considerable portion of the free yolk are at first seen in the entoderm.

The gradual increase in size of the entoderm cells, while ingesting the yolk on the median side of the embryo, at the same time forces the latter outward until that portion of it which from now on is to be recognized as the anterior end comes to

lie in contact with the wall of the capsule (fig. 17). This migration of the embryo from an interior to a peripheral position is a step characteristic of all the species considered in this paper. According to Bresslau the embryonic cells migrate to the ventral surface of the yolk in the species of Mesostomum and Plagiostomum which he studied. Hallez ('09) finds it assuming an antero-ventral position in Paravortex cardii. The same migration was observed in the Dendrocoeles by Mattieson ('03) and in the Triclads by Jijima ('84). The embryonic mass of Paravortex gemellipara, like that of P. cardii, is forced to the anterior side of the yolk.

There is considerable variation in the amount of yolk absorbed by the primary entoderm (compare figs. 18 and 20). Hence there remains a larger or smaller quantity disposed, as seen in a median section (fig. 17), in the form of a bi-concave lens between the two embryos. The fate of this mass will be discussed in the following section.

Some of the entoderm cells seen in figure 21 have already reached the limit of their absorptive capacity, while others are still comparatively small. It should be pointed out, however, that there is a great variation in size at the time they are taken into the embryo. That the quantity of yolk absorbed depends rather upon the activity of the cell than upon the amount of yolk in its neighborhood is suggested by the fact that small primary entoderm cells are enfolded by the gastrulating embryo even when there was plenty of material present, as indicated by the quantity later found in the ectoderm.

At the completion of the yolk absorption, and before gastrulation, degeneration of the nuclei of the primary entoderm cells sets in. As represented in figure 21, their chromatin becomes less distinct, while the nucleoli disappear. Finally, with the dissolution of the nuclear membranes, there remain for a time only indistinct vestiges of nucleoplasm with a little chromatin. Rarely does any trace of the nucleus appear in the cell after it has been taken into the body of the embryo. The activity of the cell having ceased, the presence of the nucleus is no longer necessary.

6. Ingestion of the yolk-gorged primary entoderm cells by those of the secondary entoderm

A remarkable event now happens. Some of the derivatives of the secondary entoderm cells pointed out in figures 14 and 16 become amoeboid and make their way around and between those of the primary entoderm until they come to lie, in some cases, next to the free yolk. Three of these are visible in each embryo of figure 18. Though the nuclei are always prominent, it is only in well-preserved and favorably stained material that the cytoplasm can easily be distinguished. In such cases there can be no question of a syncytium here. Each cell is a unit.

Another amoeba-like character is now manifested by these cells; each primary entoderm cell is ingested by a secondary entoderm cell. Referring again to figure 21 we find that the section passed through the nuclei of several secondary entoderm cells, while the cytoplasm of each stretches in a thin sheet over the surface of the primary entoderm cell which is being engulfed. There is in this embryo no certainty that the cytoplasm has yet completely surrounded the yolk-filled cells. That this occurs later will become clear. It is evident that the nuclei of the primary entoderm cells do not entirely disappear until after ingestion.

7. Gastrulation and absorption of yolk by the ectoderm cells

If by the process of gastrulation is meant the infolding or overgrowth of the entoderm by the ectoderm, then a true gastrulation occurs in Paravortex gemellipara. Bresslau ('04) in describing the development of Mesostomum thought the term inapplicable. It is used in this paper as expressing the process at least homologous with gastrulation.

The first step consists in the differentiation of the ectoderm. The outermost cells of the mes-ectodermic mass at two points (or perhaps in a ring) on the anterior surface of the embryo now become flattened. Between these two points lies a pair of cells which are to give rise to the lining epithelium of the pharynx

(figs. 22 and 23). Just as Bresslau finds in Mesostomum so in
P. gemellipara the ectoderm cells stretch backward in a sheet
over the underlying mass (fig. 21). Figure 22, drawn from a
section so thick that only the upper third of the embryo was
removed, represents the condition just before the close of gas-
trulation. The five yolk cells, three of which show the large
nuclei and thin cytoplasmic sheets of the secondary entoderm
cells whose food they have just become, are being folded into
the embryo by the overgrowth of the ectoderm cells. At the
right three of the latter are of such a character that they resemble
the yolk cells just described.

It is apparent from the above account that gastrulation in
P. gemellipara is brought about by a process of epiboly or over-
growth of the entoderm by the ectoderm cells. The latter
are formed by differentiation of the external portion of the mes-
ectodermic mass, whereby the cells, first at the anterior end,
then gradually those lying more and more laterally, become
flattened and push backward over the entoderm.

At this point another phase in the nourishing of the embryo
is observed. It was noted above that, after the gorging of the
primary entoderm cells, more or less yolk remained in the capsule
(figs. 18 and 21). The ectoderm cells, now undertaking the
role of nutrition, absorb this free yolk. In figure 22 the cells
on the right still show the yolk granules distinctly, indicating
that the process here is similar to that carried on by the entoderm.
Very soon, however, the yolk undergoes a change. The flakes
and granules in each cell flow together into a single large globule.
It is evident from this figure that the posterior cells take in most
of the yolk, those at the anterior end coming in contact with
only a very little. The nuclei of the ectoderm are characterised
by the presence of large and distinct nucleoli.

It is strikingly evident that in embryos of corresponding stages
of development, particularly that shown in figure 24, the ecto-
derm yolk-content of some is decidedly less than of others. The
conclusion is that the primary entoderm absorbed the greater
part. According to this hypothesis but little yolk would have

entered the ectoderm cells of the embryo pictured in figure 18. Such individuals as those in figures 22 and 23 must previously have had left over after the absorptive action of the entoderm an amount of free yolk comparable with that shown between the embryos of figure 21.

a. *The embryo at the close of gastrulation.* The picture presented by the embryo at the close of gastrulation is shown in median frontal section by figure 23 which was drawn from an Ehrlich's haematoxylin-eosin preparation. Sections of seven primary entoderm cells occupy the greater part of the young worm, each lying inside an amoeboid secondary entoderm cell. No clear regions have yet appeared in the interior of the former; the yolk globules are evenly distributed.

A similar condition exists in the ectoderm, although a slight advance is evidenced by the tendency of the small globules to fuse into larger masses. The posterior cells are so engorged that their membranes are stretched almost to the point of rupture. On the other hand the anterior ectoderm cells are flattened and contain only a few yolk granules. It is probable that certain large nuclei just beneath the ectoderm in this region are soon to be added to it as parts of new cells.

The formative mass from which are to be derived all the definitive organs lies toward the anterior end of the embryo. Two lines of cells, however, are pushing posteriorly beneath the ectoderm and the primary entoderm. Certain of these are undergoing mitosis, while a few differ from the majority in possessing lighter cytoplasm and definite boundaries. These are regarded as secondary entoderm cells which did not migrate outward to ingest the yolk-laden cells, but were reserved to take a part in the formation of the intestine.

It is impossible in the section from which figure 23 was drawn to detect cell membranes between the smaller nuclei which characterize the greater portion of the undifferentiated mass. One might well regard it as a syncytium were it not that the membranes are discernible in figures 18 and 21 which represent slightly younger embryos, and in figure 24 of one somewhat

farther advanced. These three figures are from preparations
stained with iron-haematoxylin and eosin after fixation in
Flemming's solution.

To conclude the description of the stage seen in figure 23, the
pair of cells mentioned above (fig. 22) as being reserved during
the early period of ectoderm differentiation for the development
of the internal pharyngeal epithelium, are conspicuous at the
anterior end of the embryo. Their cytoplasm remains con-
sistently lighter and clearer than that of the adjacent interior
cells. Their resemblance is rather on the side of the ectoderm.
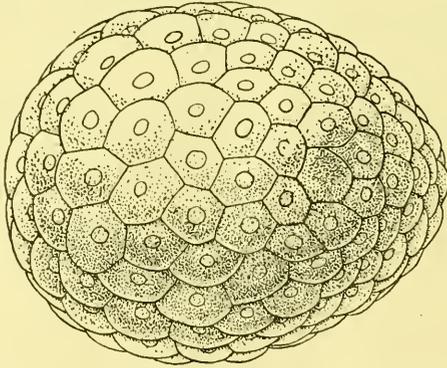
Text fig. 15   Surface view of an embryo forcibly freed from its capsule shortly
after gastrulation.   Anterior end at the right.   × 530.

Figure 25, drawn from a fresh capsule, shows the appearance
of a living embryo at a stage slightly later than that in section
(fig. 23). Through the transparent ectoderm can be seen the
prominent yolk-filled cells of the primary entoderm. The
highly refractive bodies so conspicuous in the ectoderm cells
are regarded as globules of the same nature as those constituting
the free yolk still remaining in the capsule. The larger opaque
masses in the posterior ectoderm cells are the homologues of
those noted above in figure 22, i.e., masses of yolk undergoing
digestion.

Between the capsular shell and the enclosed embryos and
yolk granules is a clear zone filled with a colorless fluid. Prob-

ably a large part of this clear region is occupied by a f uid of a watery nature which has been absorbed from the parenchyma. It was pointed out above that capsules which are freed in sea water immediately swell on account of pressure due to water absorption.

8. Organogenesis to the time of birth

a. *Origin and development of the eyes.*   The first organs to be differentiated from the mes-ectodermic mass in Paravortex gemellipara are the eyes.   Although at first this seems rather
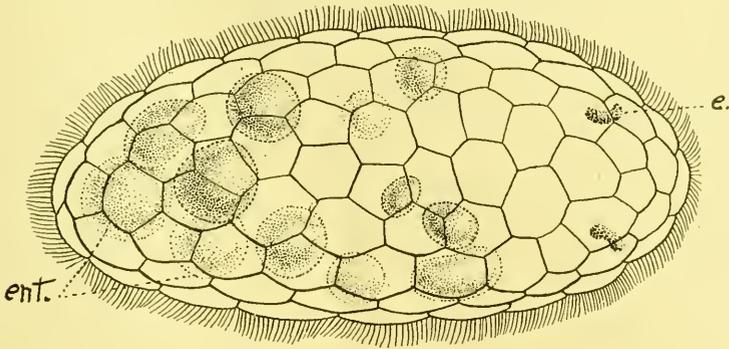
Text fig. 16   Surface view of an embryo which of its own account had made its way into the mother's parenchyma.   *ent.1.*, digested remains of yolk in primary entoderm cells; *e*, eye.   × 400.

remarkable, still it is reasonable to expect that, since the major portion of each definitive eye consists of a single cell, this cell must at an early stage take on a different appearance from those about it.   Already in figure 24 the eye cell shown has become distinct by its larger size and clearer cytoplasm.

These eye cells appear, one on either side toward the dorsal side of the undifferentiated mass.   That seen in figure 24, an oblique frontal section, is an individual cell with a distinct membrane (Hallez was unable to distinguish the membrane in P. cardii) and nucleus.   The latter at first lies in the center of the cell, but soon assumes an eccentric position toward the posterior median side.   The presence of only a single melanin granule is certain on account of the fact that the whole cell is included in

this section. Moreover, the second eye of this embryo was in the same stage.

The fate of these undifferentiated cells will be treated later. The eyes are at first separated from the ectoderm by three or four rows of cells. Partly as a result of increase in size which the eyes undergo, and partly because the cells among which they lie are gradually withdrawn to form other tissues, the eye cells shortly assume a position so close to the ectoderm that only one layer of nuclei intervenes. The left eye in figure 28 (from a frontal section through the eyes and brain) has become finally located. Apparently at this stage the optic cells are entirely independent of the brain. Unusual turgidity characterises the embryonic eye; it retains a spherical form in spite of the pressure which must be exerted upon it by the surrounding cells.

A series of stages in the development of the eye is illustrated by figures 30 and 31. All were drawn with the aid of a camera lucida in order to bring out the gradual increase in the size.

During the period of greatest activity of the eye cell its nucleus is prominent, with a fine chromatin reticulum; a nucleolus has not been detected. It has been mentioned in the section dealing with the cleavage of the egg that the active blastomere nuclei tend to assume an amoeboid form. So in the eye cell the nucleus becomes to a lesser extent irregular during the deposition of melanin granules.

Two types of melanin spherule deposition have been observed. The more usual is that wherein the granules arise one at a time. While those first formed are increasing in size new ones appear in the cytoplasm (fig. 30). The earlier melanin bodies are formed close to the lateral surface of the nucleus. Hence it might be inferred that, being laid down under the influence of the latter, these would soon be pushed outward by new ones to be formed in the same proximity to the nucleus. Such a sequence, however, has not been observed; the new spherules arise as often on the outside of the group as on the inner nuclear border. Indeed, after the process has gone on for some time practically all the smaller granules are seen to lie on the side opposite the nucleus.

As to the general arrangement exhibited by this group of melanin spherules, the early irregularity soon gives way to that of a cup whose concavity faces outward (fig. 30 *d, e*). Consequently a meridianal section is crescentic. The floor of the cup, lying against the nucleus, consists of from two to four rows of larger granules, while as the rim is approached the thickness is gradually reduced to one small sphere.

According to the second type of melanin deposition many minute particles arise simultaneously in the cytoplasm between the eccentrically placed nucleus and the outer membrane of the eye cell. Figure 31 represents the appearance and history of these granules. Since the final arrangement is the same as in the eye at the end of the series described above, it appears either that the spherules nearest the nucleus increase more rapidly in size while those at the margin of the cup remain consistently smaller, or that new granules have subsequently been added to this rim. The latter hypothesis is supported by the fact that a larger number of spheres is present in the definitive eye than was first seen to arise.

It seems clear that the pigment granules arise in the cytoplasm, probably under the influence of nuclear energy. Their arrangement and appearance are not visibly affected by the method of preparation; hence they can be favorably studied in preserved material. When first detected each is a tiny homogeneous refractive body whose form is that usually assumed by a drop of fluid whose surface tension properties are different from those of the fluid in which it is suspended—in this case cytoplasm. There is no evidence that these spherules enlarge by the accretion of successive layers of a more solid material. The conditions rather favor the idea that they grow in the manner observed in the case of fat drops, i.e., by an accumulation of more and more fluid.

With the increase in size the yellowish color deepens to a reddish brown, and a darker central dot appears in each granule (fig. 30 *c*). From this point on there is little to be noted beyond the increasing opacity due to the greater amount of material. Finally, when the pigment spheres have reached their full size

their color is an intensely dark brown (fig. 30 e).   At low magnifications the pigment cup is to all appearance black.

The nucleus of the eye pigment cell is gradually forced by the melanin granules against the membrane at the postero-median side.   Figure 32 shows it thus flattened.   In proportion to the size of the cell the nucleus is now smaller than during the period of its greatest activity.

b. *Development of the brain and larger nerve trunks.*   Bresslau ('04) determined that the formative mass in the embryo of Mesostomum ehrenbergi soon separated into three anlagen, a large antero-ventral mass, consisting at first of two ganglia which later formed the brain; a spherical portion just posteriorly from which the pharynx musculature arose; and third a smaller cresentic gonad anlage.

Hallez ('09) noted the similarity exhibited to this stage of Mesostomum by the corresponding stage of Paravortex cardii.   In the development of P. gemellipara the differentiation of the brain, pharynx musculature, and gonads is essentially the same as in the species studied by Hallez.   In two laterally symmetrical regions toward the dorsal side of the anterior embryonic mass a proliferation and grouping of nuclei occurs soon after gastrulation.   Thus the two ganglia of the brain arise.   While the nuclei become more and more numerous, it has been impossible to discover any mitoses in this region, a fact which led Hallez to suggest that division here is amitotic.

The second feature noted in the development of the brain is the appearance simultaneously of two clearer protoplasmic areas in the center of each lateral ganglion.   The substance occupying these two regions has been called 'Punktsubstance' by Leydig. As pointed out by Bresslau and Hallez, these two centers soon fuse to form a transverse dumb-bell shaped core on the outside of which the ganglion cell nuclei are arranged.   The latter are at first indistinguishable from those of the surrounding portions of the undifferentiated cell mass, but in slightly older stages are visibly smaller (figs. 29 and 53).

Concerning the 'Punktsubstance' mentioned above one characteristic is to be noted that neither Bresslau or Hallez described

in either text or figures, namely, the striated or fibrillar structure of the lighter brain core when viewed in sagittal sections. That these striations are to be regarded as the fibers or prolongations of the ganglion cells whose nuclei are distributed over the surface of the brain, is clear from the works of many investigators. These structures are well represented in Bronn's Klassen und Ordnungen des Thier-Reichs. Obviously it is these fibers which, in a cross-section of the brain, i.e., a sagittal section of the worm, are responsible for the dotted appearance. Figures 29 and 37 are of sections so cut that some nerve fibers are seen in cross and others in longitudinal section.

Little remains to be added in regard to the nervous system. Since the material was not treated with differential nerve stains, it is extremely difficult to follow the nerve trunks through the mesenchyme, even in older specimens.

Concerning the origin of the two posterior nerves of Paravortex cardii Hallez says:

"Quant aux deux troncs nerveux postérieurs, tout ce que j'ai pu voir, c'est qu'ils se différencient *in loco*. . . ."

It is believed that in P. gemellipara these posterior nerves can hardly be said to arise '*in loco*.' They evidently push backward through the mesenchyme from the posterior angles of the brain. In figure 28 the nerve fibers can be traced thus for a very short distance, but in a later stage (fig. 29) the ends have reached farther toward the posterior end.

It is important to notice that many ganglion cell nuclei are carried backward along the nerve trunks. Consequently the cephalic mass suffers a reduction. From the conditions observed it is concluded that in the Turbellaria, as in the vertebrates, the nerve fibers, with groups of ganglion cells, originate in the region of the brain and migrate toward the periphery.

That the optic nerve trunks have a similar origin may be argued from the appearance presented by figures 28 and 37. However, these nerves may have another origin. The fibrous brain core in figure 28 has begun to stretch forward at two antero-lateral points; a decided advance has taken place at the stage pictured in figure 32. Here the left lobe of the brain and the

left eye are viewed from the dorsal surface. The nerve fibers show very plainly passing from the 'Punktsubstance' region of the brain outward and between the ganglion cells. The course of the nerve is then directed laterally around the posterior side of the pigment cell to its external surface.

At this point the eye cell, whose origin and growth have already been described, undergoes an invagination. This is apparently effected by the pressure of a nerve cell which is seen lying in the concavity. This undoubtedly will form the 'sight-cell' or end organ which is stimulated by light rays. A second nerve cell nucleus is observed just posteriorly.

It can now be suggested that the two cells lying close outside the pigment cell in figure 32 arose in this position and have sent fibers backward to meet the anterior extension of the brain. Two facts support this view. First, undifferentiated cells during development lie in this region between the ectoderm and the pigment cell. Secondly, it is well known that in higher forms the cell body of the receptor is located in the retina, while the axone extends into the brain. It is obvious, however, that one must not attempt to homologise too closely the course of development of a turbellarian with that of a vertebrate. In the latter the retinal cells are first carried out by the optic cup from the central nervous system, while in the Turbellaria the boundaries of cell groups in the cephalic region are notably indistinct.

It has proved impossible to discern in my material the structure of the cell or cells which occupy the external invagination of the pigment cell in the adult worm. Since the histology of the eye in related Turbellaria has been determined, it is to be expected that the receptor of P. gemellipara is similar to that observed, for instance, in Phaenocora and Mesostomum as shown in Bronn's Klassen und Ordnungen des Thier-Reichs.

Figure 33 of this paper was drawn from an adult eye of P. gemellipara, and shows that the optic nerve, as in the embryo and in the species to which we have just referred, passes to the lateral invaginated surface of the pigment eye-cell. A nucleus rests close outside, apparently in the nerve.

c. Pharynx. Simultaneously with the differentiation of the brain the anlage of the pharynx musculature becomes marked off from the former and from the remaining tissue by a closer grouping of larger nuclei. This mass lies ventral and very slightly posterior to the brain, but its boundaries are at first so indefinite that, were it not for the simultaneous ingrowth of the cells which form the internal pharyngeal epithelium, the anlage would with difficulty be distinguished.

Considerable attention has been paid by European observers to the development of the pharynx of the Turbellaria. Mattieson in 1904 described the process in the Dendrocoele, Planaria torva, in which the pharynx arises as a spherical mass of cells on the ventral surface just beneath the primary ectoderm. While the outer cells form the musculature of the organ, the internal epithelium arises from a few large cells situated on the interior. Against the inner end of the pharyngeal mass lie four or five cells which in Mattieson's opinion are entodermic in nature. No vestibule intervenes between the pharynx and mouth.

Bresslau ('04) found that in the Rhabdocoele Bothromesostomum personatum the internal epithelium likewise arises *in loco*. In Mesostomum ehrenbergi, a different condition exists; the lining epithelium is derived from a rod of cells which grows inward from the ectoderm and pierces longitudinally the mass which eventually becomes the pharyngeal musculature with its glands. After traversing the pharynx the ectodermic rod acquires a lumen and gives rise at the inner end to what Bresslau terms the inner pharngeal pouch lying between pharynx and yolk, while between the pharynx and mouth an outer pharyngeal pouch has a similar origin.

The main features in the development of the pharynx in Paravortex gemellipara agree with those described by Bresslau for Mesostomum ehrenbergi. Until after the ingrowth of the epithelial rod no definite grouping of nuclei occurs to mark the origin of the pharyngeal bulb. The cells which are to take a part in its formation lie loosely arranged in the mid-ventral portion of the undifferentiated cell mass just beneath and posterior to the brain.

In the description of the ectoderm formation there was pointed out a pair of cells which, although at that time lying at the anterior surface of the embryonic mass, did not enter into the development of the body covering. These two cells, well shown in figure 23, together with two or three others not seen in this section proliferate the rod of cells which, as in Mesostomum, grows inward to penetrate the mass which lies beneath the brain.

An intimate connection between these cells and those of the ectoderm is evident in figures 34 and 26. The former represents a section which is obliquely sagittal. It is evident that before the cells of the pharyngeal bulb have become closely grouped, the posterior cells of the epithelial rod have penetrated the mass of dark nuclei and lie against the entoderm. A distinctly clearer cytoplasm and paler nuclei serve to distinguish them from the neighboring cells. Each of their nuclei, like those of the ectoderm, with whose early history these cells are closely associated, contains a nucleolus. Externally a slight invagination of the ectoderm indicates the future position of the mouth.

A slightly later stage is shown in figure 27, a transverse section through the posterior lobes of the brain and the center of the pharynx. Portions of seven clear cells belonging to the epithelial rod are conspicuous in the center of the mass of nuclei which have now become closely grouped with their cytoplasm in what appears to be a syncytium. For a longitudinal view of the organ at this stage the obliquely frontal section in figure 26 is of interest. The nuclei of the bulb are disposed roughly in two rows, somewhat toward the periphery of the mass. As yet no cell membranes appear between them. The epithelial cells are constricted within the bulb so that they become elongated, parallel with the longitudinal axis of the organ; one cell appears to traverse its entire length. Those between the pharynx and epidermis are irregularly arranged, while at the inner end of the pharynx is a group of five similar cells. Three of these appear to be intimately connected with the epithelial rod; the posterior pair, however, are not thus closely associated, but have, it is believed, an entodermic rather than an ectodermic origin. Further

notice of these cells will be taken when the development of the intestine is considered.

In an older embryo (fig. 37) the muscular portion of the pharynx has become sharply defined, although no external epithelium has yet developed. The nuclei have not migrated farther toward the periphery than the point at which they were observed in the preceding stage. A considerable enlargement of the anterior and posterior cells of the epithelial rod, has occured. No blind sac, however, is formed, as in Mesostomum, at either end of the pharynx. Indeed, no lumen appears in the pharyngeal apparatus until shortly before the embryo leaves the mother's body.

If at this stage the pharynx and associated tissues of Paravortex gemellipara be compared with the corresponding stage in Mesostomum ehrenbergi it is evident that the development of the pharynx in the former lags behind that in the latter. Mesostomum shows a well defined epithelium bounding a large cavity, the latter consisting of an internal and external pocket communicating by a tube through the pharynx. Bresslau considered the interior pocket as the esophagus, whose cells differed in point of origin from those later forming the intestine. The homologue of this pocket in P. gemellipara is the group of large cells just posterior to the pharynx.

In the cross section of this organ represented in figure 38 an advance is shown in the presence of an external epithelium. A thin layer of plasma containing a few small flattened nuclei covers the pharyngeal bulb. Bresslau derived this tissue from the outermost portion of the pharyngeal musculature anlage in Mesostomum; but in Paravortex the indications point to a mesenchymatous origin, such as von Graff ascribed to it in other Turbellaria. When one remembers that in either case the cells constituting this membrane arose in the undifferentiated cell mass the point resolves itself into one of time.

Concerning the development of the radial, circular and longitudinal fibers which arise in the pharyngeal musculature nothing has been observed in the young of Paravortex gemellipara while still in the mother's body.

d. Musculature.   Like the nervous system and the pharynx the mesenchyme and dorsoventral muscle cells arise from the undifferentiated mass which occupies the anterior portion of the embryo at the close of gastrulation.   If one compares figure 23 and figure 24 it is noticed that in the latter indifferent cells have migrated posteriorly so that a continuous sheet of them lies between the ectoderm and entoderm.   Their amoeboid nature is further evidenced by the extreme delicacy of their membranes. Indeed, it is impossible to distinguish a boundary where two cells come in contact with each other.   The tissue at this stage may be called a syncytium.   Occasional mitoses are observable in this region.   The plane of the section of the embryo at the right in figure 24 is sagittal, and somewhat toward the left side of the body, and the dorsal margin lies toward the top of the page.   Thus it appears that in P. gemellipara the migration of cells occurs as freely in the dorsal as in the lateral and ventral portions of the embryo.   In the later stages, however, as Hallez pointed out, there are comparatively few nuclei on the dorsal side of the entoderm (fig. 37).

There are two types of nuclei in this sub-epithelial layer, an external row of small rather deeply stained nuclei which usually lie against the inner surface of the ectoderm cells, and an internal series of larger and paler nuclei, some of which are slightly irregular.   The second type is more numerous on the lateral and ventral surfaces.   The embryo at the left was tangentially sectioned, thus showing the more lateral nuclei.

Passing to the more advanced stage shown in the sagittal section in figure 37, where the brain and pharynx have become differentiated, it is found that certain of the cells containing the smaller nuclei have become spindle-shaped.   The cytoplasm has formed a process at each end.   These spindle cells are still more conspicuous in figures 27 and 36.

Bresslau ('04) describes similar cells at a corresponding period in the development of Mesostomum as representing the early stages in the differentiation of the dorso-ventral muscle fibers. The number observed just beneath the ectoderm of Paravortex rather suggests that from these cells arise the circular and longi-

tudinal muscle fibers which soon become so conspicuous in this position (fig. 40). These sub-epithelial muscle cells of the adult Rhabdocoeles have been observed by many investigators. The figures in Bronn's "Klassen und Ordnungen" show the remarkable extent to which they become fibrillated.

In Paravortex as in most of the Rhabdocoeles, a muscle net is developed just beneath the epidermis. Figures 40 and 41 show the outer layer of circular and inner layer of longitudinal fibers in the adult condition. Since the figures in the various works which have been accessible never show nuclei in connection with the fibers of this muscle net, a vigorous attempt was made to find the cells from which these fibers arise in the embryo of Paravortex gemellipara. Of interest in this regard is the cell shown in figure 39, for it is possible to trace several long delicate fibers outward from the cytoplasm surrounding its nucleus. The latter lie close beneath the external epithelial cells in the position later occupied by the muscle net. Coe ('99) traced such a development of the muscle fibers in the Nemerteans Micrura caeca and Cerebratulus marginatus.

If this interpretation of the origin of the muscle fibers be correct, then a further question presents itself. Do these fibers later become independent or do they maintain a connection with such a cell body as is seen in figure 39? It is difficult to conceive, judging from the parallel arrangement of muscle strands in the adult, that the fibers are still in connection with these cell bodies. The latter, moreover, cannot be detected in the adult tissue. The conclusion is, therefore, that either the fibers become separated from the nuclei, or that the latter by repeated division are so reduced in size that they escape observation.

e. Sex organs and vitellaria. Not until after the young leave the mother's body does the true differentiation of the sex organs commence. At the time the young worm breaks out of the capsule and enters the surrounding mesenchyme the anlagen of the two hermaphroditic glands are only loose masses of cells, one on either side just lateral and posterior to the pharynx. In one animal which had forced its way into the mother's intestine a few cells of these paired anlagen were undergoing mitosis,

while those of the neighboring tissues were entirely without mitotic figures (fig. 49).

Just previous to this stage of development occasional large cells make their appearance in the ventral region of the body between the entoderm and the layer of muscle fibers. These cells are represented in figures 37 and 43 as having larger, clear nuclei and a considerable amount of darkly stained cytoplasm. The color of the latter in iron-haematoxylin preparations is a bluish gray.

It is believed that these large cells later develop into the yolk cells of the vitellaria. The grounds for this conclusion will be brought forward in the section on post-embryonic development. But their behavior while the young worm is still enclosed in the capsule may be traced somewhat farther. Since they appear first in the region close behind the sex organ anlagen it is probable that they arise, either directly from them or from cells which have had a similar ancestry. It is known of other Turbellaria that the vitellaria become differentiated from the same mass with the ovaries and the testes (Hallez '09). So in Paravortex gemellipara, which at the height of its reproductive activity is characterized by the presence of remarkably extensive vitelline glands, the general rule holds concerning their origin; but in point of time their branching off from the common sex organ mass seems unusual.

f. Cilia. The epithelial cells become furnished with cilia shortly after the stage seen in figure 34. They are already present in figure 35, but of their development nothing has been observed beyond the fact that, although they are as long when first noted as in older worms, they are not nearly so numerous as on the adult epithelium. The embryos at this stage are able to move about in the capsules. The ciliary action can easily be observed through the maternal tissues when the living worm is studied.

g. Embryonic digestion and early development of the intestine. In an earlier section of the paper it was explained that the gastrulating embryo, like those of all Rhabdocoeles, enclosed within itself the free yolk material which had previously entered the capsule as a constituent of the vitelline cells. The greater

portion was absorbed by primary entoderm cells which were in turn ingested by amoeboid secondary entoderm elements. The ectoderm grew backward over the entoderm and at the same time absorbed the remainder of the vitellarial yolk. Figure 23 was pointed to as illustrating the appearance of the embryo at the close of gastrulation. By far the greater portion of the embryo is occupied by the yolk-laden cells.

The fate of this yolk now remains to be described. In the stage represented by figure 23 little change has occured in the nutritive material. It is of the same nature as the free yolk seen in figure 21, with the exception that the flakes and globules are less closely packed in the cytoplasm of their containing cells. There is observable, however, a tendency of these particles to flow together into larger globules, a process which is more rapid in the ectoderm cells. Thus in the two epithelium cells at the top of figure 23 several such spheres are conspicuous.

In an older embryo (fig. 34) this sphere formation has advanced considerably. The spherules of nutritive substance combine again and again until the major portion is included in one or two immense globules. That the yolk is undergoing a transformation is indicated by the appearance of darker spherules suspended in the large masses. These smaller elements stain dark brown or black in iron-haematoxylin preparations such as figure 34 represents.

In figure 25 is illustrated the appearance of the ectodermic nutritive material in the living embryo after treatment with the neutral red stain. The latter has a more rapid effect upon the larger masses than upon the smaller. After immersion for a very few minutes in a dilute neutral red solution it is impossible to see through the posterior ectoderm; all is a mass of deeply stained red spheres.

Returning to figure 34 it is seen that clear regions have appeared in the interior of each entodermic yolk cell. These regions in the iron-haematoxylin-eosin preparations are perfectly colorless, but in those treated with Ehrlich's haematoxylin and eosin after fixation with corrosive acetic, they take the eosin color (figs. 27, 36). Thus it is evident that these lighter portions are

filled with a plasma. To how great an extent the separation of the yolk-filled contents into two zones is due to the action of reagents has not been determined. It is interesting to note in this connection that most of the spherical yolk-mass enclosed in the embryo in figure 42, an Ehrlich-stained specimen, is homogeneously filled with yolk-granules such as originally entered the cells. Still this embryo, as indicated by the presence of the cilia, is more advanced in development than the one illustrated in figure 34.

One entoderm cell, that at the upper left in figure 42 demands attention. The yolk material which it contains has entirely assumed the fluid nature seen in central regions of these cells (fig. 36). Now it has been explained that the Flemming-iron-haematoxylin-eosin method leaves these regions entirely colorless. Hence the cell now under consideration, if stained by this latter method, would have apparently contained a vacuole in the position here occupied by the yolk mass. Exactly such a condition is exhibited in figure 37. In this embryo there appear what at first were regarded as vacuoles within a mesenchymatous network; they are now rather to be interpreted as the transformed yolk material accumulated within the entoderm cells as immense globules which, following the corrosive-acetic Ehrlich's haematoxylin-eosin method, stain a rich yellow. It has been pointed out that by this method also the nuclei, cytoplasm and membranes of the entoderm cells containing these nutritive masses are much more clearly stained than by the iron-haematoxylin.

Another feature to be noted in the entodermic yolk masses in figure 42 is that the mitochondrial mass is again discernible. This body was traced from its origin within the oocyte through cleavage to its division and enclosure in the primary entoderm cells. During the early history of the mass the Ehrlich stain was unfavorable to its demonstration, since the cytoplasm of the cell retained so deep a stain that the mitochondrial mass was obscured. But in the late stage shown in figure 42 the cytoplasm of the original cell has broken down and the granular mass of mitochondria again comes into prominence. The centrosome

with which it was earlier associated may still be detected, al-
though less easily than during the period of its activity.

A certain fact favors the view that a process of disintegration
has attacked the mitochondrial substance. For whereas, up
to the time when the entoderm cells had absorbed a considerable
amount of yolk, the body appeared conspicuously in iron-
haematoxylin material, it has been found impossible to detect
it in such preparations at later stages of development. This
peculiarity, taken in connection with the ability of the Ehrlich
stain to bring it out subsequently, points to the conclusion that
the mitochondrial substance has become chemically changed.
Furthermore, no trace of it is to be observed after the yolk
globules have been transformed into such a fluid mass as noted
in the upper entoderm cell of figure 42. It would be too extreme
to surmise that the mitochondrial mass represents a substance
laid down in the oocyte for the purpose of acting as a digestive
agent after the yolk has been taken inside the embryo. Such
an interpretation would have to regard the substance as a poten-
tial ferment, which, before it could act upon the yolk, must
undergo a transformation which renders it invisible.

Against the view that this mitochondrial mass is functional
at this stage is the evidence furnished by the ectoderm. Here
a simultaneous yolk-digestion has been in progress where none of
the mitochondrial substance entered. In figures 35 and 37
each posterior epithelial cell contains one or more of the large
fluid spheres. In appearance and reaction to the Ehrlich's
haematoxylin-eosin stain, these masses are closely similar to those
in the entoderm. As we have seen, their origin also is the same.
In the iron haematoxylin preparations, however, the ectodermic
material stains brownish while the entodermic masses are color-
less. It is probable that both substances are lipoids of some
sort.

After the young worm has left the capsule and is prepared to
take food through the mouth the reserve material present up to
this time in the ectoderm rapidly disappears. The process is
apparently one of gradual absorption and assimilation. In
one preparation a cell just beneath and closely applied to the

ectoderm contained two small globules, of the same consistency and color as the food material in the entoderm. It is possible that through the agency of mesenchymatous cells the ectodermic nutriment is passed on to the tissues of the embryo. On the other hand this cell may have belonged to the entoderm, its contents representing the last stage in the digestion of the entodermic yolk.

During the absorption of the reserve food, the ectoderm, or external epithelium of the young worm, has undergone vacuolization; the cytoplasm of each cell now forms trabeculae between irregular clear regions (fig. 37). This condition is at first more noticeable at the anterior end of the body where less yolk is deposited, but with the absorption of the latter posteriorly the whole ectoderm takes on this appearance. This vacuolization causes a marked increase in the depth of the cells.

h. Formation of the intestine. According to Bresslau ('04) the intestinal epithelium is formed in Mesostomum by previously undifferentiated embryonic cells which unite first in the region just posterior to the esophagus. Gradually this sheet of cells, by the addition of others, extends posteriorly about a cavity which arises as a splitting of the tissues in the region of the fast shrinking yolk cells. At the same time the intestinal cells become vacuolated.

Hallez ('09) asserted that the cells which enter into the formation of the intestine of Paravortex cardii are of the same nature as those which give rise to the mesenchyme. He considers their immediate predecessors to be large cells lying at the posterior end of the body. These proliferate at once cells which are added to the intestinal 'syncytium,' and others which constitute the mesenchyme between the intestine and body wall. He observed that a lumen appears first in the region just behind the oesophagus, and gradually, with the absorption of the vitelline spheres, extends posteriorly.

From certain observations of the conditions under which the intestine arises in Paravortex gemellipara' there is some doubt that it originates in the manner described by Hallez for P. cardii. It seems rather that the large posterior cells, as noted in the

preceding section on the sex organ and vitellarium anlagen, ultimately differentiate to form the vitelline cells. Their development will be considered later.

In P. gemellipara the intestine is organised from cells which, at the stage illustrated by figures 35 and 36, are proliferated in the region just posterior to the oesophagus. Two of these in figure 26 were pointed out above while the pharynx was being considered. It is believed that they are derivatives of the large clear cells designated as *ent. 2* in figure 23.

With the rapid absorption of the yolk inside the secondary entoderm cells, the latter become enclosed with the epithelial cells which, arising in the manner above described, migrate backward about and between them. In this way a loose rod of cells comes to occupy the whole region behind the pharynx. As Hallez observed, the intestinal lumen first appears at the anterior end of the rod, and gradually extends posteriorly. As long as the young worm remains in the capsule there is no communication with the outside through the pharynx and mouth. It is probable, however, that it is potentially present, requiring only the muscular activity of the pharynx to open the lumen. The young animals, when liberated from the mother by pressure upon the cover-slip, exhibit contraction and expansion of the pharyngeal apparatus, a feature which indicates that, as soon as they are in a position to obtain food from without, they are provided with a lumen from mouth to intestine.

i. Glands. Shortly before the young worm leaves the capsule from two to four elongated oval or spindle-shaped bodies appear between the brain and the vertex of the anterior end of the body (fig. 45). In iron-haematoxylin preparations these masses are grayish in color, while Ehrlich's stain imparts to them a deep red. In animals which have entered the tissues of the mother these gray bodies communicate with the exterior (fig. 46) by slender processes; they are now recognizable as the cephalic glands. To these has been attributed the function of secreting a slime which lubricates the animal's path.

Numerous other spindle-shaped cells distributed through the sub-epithelial tissue are evidently single-celled glands which have to do with the secretion of mucous.

9. Post-embryonic development

In order that the further development of the sex organs, intestine, mesenchyme and glands could be studied, an attempt was made to keep alive in small aquaria young worms which had been removed from adults, into whose parenchyma they had already become freed. No success was met with; all died inside of seventy-two hours.

Therefore several young of the smallest size obtainable from the mussels by ordinary methods were sectioned in the hope that some of the organs would be found in an undeveloped state. While this trial gave some interesting results, it is desirable that a study be made of specimens younger than these and at the same time older than those secured at the time of birth.

The following observations are submitted as being of interest in this connection.

a. *The definitive intestine.* Hallez describes and figures the adult intestine of Paravortex cardii as consisting of a veritable syncytium, a mass of cytoplasm containing large vacuoles and scattered nuclei. The intestinal wall of P. gemellipara, however, consists of elements which are distinct except at their extreme bases. Figure 47 was drawn from a worm which measured 0.30 mm. in length. The intestine, having already assumed the adult condition, is seen to consist of large cells which extend from the outer surface of the organ inward to its lumen. Their bases are constricted and narrowed, while the distal ends assume a rounded form where they extend into the intestinal cavity. Numerous vacuoles, some of them containing food materials, are distributed throughout the cytoplasm. Most of the nuclei are located in the narrowed ends of the cells. Figures 8 and 9 exhibit a similar appearance of the intestine of old worms. A like structure of the turbellarian digestive tube is figured in such works as von Graff's Monograph and Bronn's "Klassen und Ordnungen,"

b. *Mesenchyme.* According to Hallez's ('09) account of P. cardii, the mesenchyme receives elements, both from the undifferentiated cell mass at the anterior end of the body and from the large cells which become grouped at the posterior extremity.

Bresslau ('04) also derived the mesenchyme from the undifferentiated cells.

The origin of this tissue in P. gemellipara agrees with that described by these investigators except that there is no evidence of a contributory action on the part of the large posterior cells; it is all derived from the undifferentiated cell mass, from which cells migrate posteriorly between the intestine and body wall. The mesenchyme of the free-swimming young worm from which figure 47 was drawn already existed in the condition which is characteristic of the adult. The cells are large and loosely arranged with indistinct and irregular boundaries.

c. *Ovaries and testes.* The youngest stage in the development of the sex glands of P. gemellipara in which they can be recognized is shown in figure 47. Although the testis lies close to the anterior end of the ovary, the two glands are already separate. No epithelium, however, has been formed about them. Since this specimen had been stained with Ehrlich's haematoxylin, and since the section was so thick that the entire testis and nearly all of the ovary are included, the minute condition of the chromatin cannot be determined. The small dimensions of the nuclei in comparison with those observed in the adult ovary indicate that all the cells in the female gonad, except possibly the most posterior, are oogonia.

An important feature in this specimen is that the vitellaria have become conspicuous, particularly in the posterior half of the body. No connection between these glands and the ovaries can be traced in this series of sections.

The next instructive stage which my material presented is shown in figure 50, a sagittal section through one testis, the seminal vesicle, atrium, shell-gland and the tissue which is to form the antrum. In the testis the spermatogonia and spermatocytes can easily be distinguished. Hallez described and figured a similar appearance for the young testis of P. cardii. One feature of which, however, he failed to give an account is that the gland becomes provided with a flat epithelium. The latter is derived from the mass of cells lying below the mid-ventral side of the intestine. From this same mass the accessory reproductive

organs are also being differentiated. It is evident that the seminal vesicle arises from the anterior portion of the mass; only a small number of cells at the extreme anterior end go to form the vasa deferentia and the external epithelium of the testis.

In the region ventral and posterior to the seminal vesicle the atrium has differentiated simultaneously. Like those of the former, its walls are composed of two layers of cells, an inner cubical epithelium and an outer sheet of flattened cells. As yet no connection of its lumen with the outside of the body has been effected. Indeed, the cells are not even in close contact with the body wall.

From the posterior surface of the atrium a dense mass of cells leads a short distance dorsally. The antrum femininum will arise later in this position. On the ventral surface of the mass is a group of cells whose rounded posterior ends have begun to reach backward behind the atrium. These cells enlarge to form the single-celled shell-glands.

Of the oviducts nothing is as yet present but a loose string of undifferentiated cells which lead from the antrum anlage posteriorly and dorsally to the ventral surface of the ovary. It was impossible to distinguish about the latter such an epithelium as was described for the testis, nor is it evident in later stages.

The ovaries in the worm from which figure 50 was drawn are well advanced. The older oocytes have undergone a considerable growth.

Figure 51, from a somewhat larger worm, is presented to show clearly the strand-like structure of the testis and the point at which the vas. deferens enters the seminal vesicle. The oogonia occupy the anterior region of the testis. These, by growth, give rise to the large primary spermatocytes lying on the ventral and dorsal sides; while in the center of the posterior half a row of smaller, densely stained cells are regarded as secondary spermatocytes. No spermatids were distinguished with certainty, but mature spermatozoa are conspicuous in the anterior central region whence they can be traced dorsally between the cell strands to the upper surface of the testis. Others lie just be-

neath the external epithelium and in the vas deferens, while
many have already entered the seminal vesicle. Thus it is
evident that Paravortex is protandrous to the extent that sperma-
tozoa are produced before the female reproductive tract has
become differentiated, for no lumen has appeared farther back-
ward than the atrium.

d. *Vitellaria.* In the section wherein the earliest anlagen of the
sex organs were described it was noted that several large cells
with an unusual quantity of darkly staining cytoplasm and con-
spicuous nuclei were observed to arise in the region of the gonads
and to migrate posteriorly between entoderm and ectoderm
(fig. 43). While these cells became distributed on all sides of
the latter, a marked accumulation occurs at the extreme posterior
end (fig. 44). Hallez was of the opinion that these cells in P.
cardii contributed largely to the formation of the intestine, but
also to the mesenchyme and vitellaria. There is no evidence
that they give rise in P. gemellipara to any other organ than the
vitellaria. These cells are identical in appearance with the
young cells which lie at the extreme tips of the vitelline strands
in the adult. Figure 44 shows at once the embryonic cells and,
just outside the capsule, several of the young vitelline cells in
the mother's tissue.

By the time the young worm has attained the stage represented
in figure 47 strands of cells similar to those of the adult have grown
forward from the posterior mass; at the same time the scattered
cells of the same type have divided to form groups here and
there through the mesenchyme of the posterior region. At x
two of them are undergoing mitotic division; similar figures
have been observed at the tips of the adult vitellarial strands.
As yet very few have appeared in proximity with the ovaries.
Hence there seems to be no evidence that in P. gemellipara the
vitelline glands develop, even in part, from the hermaphroditic
gland as it appears at this stage; these organs lack all connection
with any other densely stained tissue. In the reconstruction
(fig. 52) from an older worm, however, the vitellaria have formed
a contact with the posterior ends of the ovaries. The various
groups of yolk cells have anastomosed to form an extensive gland.

V. DISCUSSION

In the preceding account of the development of Paravortex gemellipara little attention has been paid to the interpretation given by Hallez ('09) of the processes of yolk absorption and the origin of the ectoderm in P. cardii.

He maintains that, while the morula is being formed by the cleavage of the egg, the membranes of the yolk-cells included in the capsule disintegrate, but that a part of their nuclei remain prominent and active. Hallez finds these nuclei shortly afterward lying in a clear plasma-like portion of the yolk which has become separated from an eosin-staining constituent and has assumed a peripheral position outside of and between the embryos. On the other hand the 'eosinophile' portion of the yolk separates, he believes, because its chemical and physical properties are different from those of the cytoplasmic remainder. As these properties become more pronounced, the central yolk material splits off in the form of great drops in whose interior he finds a variable number of vacuoles. He has figured several nuclei, which are assumed to have been set free by the disintegrating yolk cells, lying in the outer clear zone; others have migrated inward between the embryos. As pointed out earlier in this paper, he found that one of these nuclei became associated with each of the 'balles vitelline.'

Even more remarkable is Hallez's account of the developmental phenomena which immediately follow. Into each vitelline sphere one of these migratory nuclei penetrates, together with a small quantity of the cytoplasm which also was previously a constituent of the vitellarium cells. At the same time the material of these yolk spheres assumes a granular appearance.

A little later these nuclei, he states, emigrate with their associated cytoplasm from the 'balles vitellines.' Part of these amoeboid cells are asserted then to mingle with the envelope of plasma outside the embryo so as to form a 'syncytium' from which a primary ectoderm soon differentiates. Other such cells delaminate from the yolk spheres as additions to the ectoderm. Into the formation of the latter now enter, as Hallez believes,

the remaining vitellarial nuclei which did not previously pene-
trate the yolk spheres.   Hallez observed also that the true embry-
onic cells contribute to the development of the ectoderm.   This
ectoderm is retained permanently.   The remaining 'migratory
cells' form another 'syncytium' about the vitelline spheres which
they have just left, this constituting, in Hallez's opinion, the
primary intestine.

The ectoderm on the apposed surfaces of the two embryos is
formed by a delamination through the center of the yolk-cell
derived plasma with its migratory nuclei.   Hallez discerned but
few cell membranes.

From a study of his figures and descriptions it seems possible
that owing to a failure to distinguish the extremely delicate
outlines in the embryonic structures he has been led to the above
interpretation.   I believe that further study may show that the
processes of early development in Paravortex cardii are very
nearly the same as those which I have found in Paravortex
gemellipara.

The derivation of the ectoderm and entoderm as observed
in the development of P. gemellipara agrees essentially with
that determined by Bresslau for the several species of Rhabdo-
coeles which he studied.   A variation in the manner of yolk
absorption, however, occurs in Paravortex.   Whereas he found
the yolk cells either to retain their envelopes, as in Mesostomum
ehrenbergi, or to be enclosed by the ectoderm in the form of
numerous yolk spheres resulting from the breaking down of the
vitelline cells, in P. gemellipara the yolk granules are first ab-
sorbed by the primary entoderm cells, and these are in turn
ingested by the secondary entoderm.   The first step of the
process is similar to that known to occur in the chick, where
the intestinal entoderm absorbs the yolk particles.

That the yolk-absorptive function of these entoderm elements
is their chief capacity is further indicated in a freak embryo
shown in figure 53.   This specimen, beside lacking the yolk-
filled masses so characteristic of normal embryos, exhibits a
remarkable distribution of the undifferentiated tissue.   The
latter has proliferated at a normal rate, so that following its

posterior migration it has become arranged many rows thick between the ectoderm and a few large clear cells in the center. The latter are believed to belong to the primary entoderm which ordinarily absorbs the yolk; in this case it is probable that no food was enclosed with the egg in the capsule.

The behavior of the entoderm cells in P. gemellipara is comparable to that observed in 1884 by Lang in the Polyclad Discocoelis. Since the egg of this worm contains a rich supply of yolk material, none is added in the form of vitellarial cells. During segmentation an upper and a lower quartet of entoderm cells arise whose derivatives later form the wall of the alimentary canal. These entoderm cells have a more or less amoeboid character, sending out protoplasmic processes over the yolk spheres. The latter were previously split off from the yolk-filled middle entoderm which takes no part in the formation of any organ.

In 1907 Surface described the development of the alimentary canal in Planocera inquilina. He found that practically all of its tissue arises from two large entoblast cells. By division they form two sorts. One group containing the yolk is pushed into the interior of the embryo where the cells break down and the yolk granules flow together into a fluid mass of large spheres. Meanwhile the lower entoblast cells divide rapidly, thus giving rise to a large group of cells. A cleft, the first indication of the intestinal lumen, now occurs in this entodermic mass, and cell outlines become distinct about the nuclei. The important feature to be noted in relation to the behavior of the entoderm of P. gemellipara is that Surface observed these cells to become amoeboid and to spread out on the surface of the large yolk spheres which are thus absorbed.

Now it is only a short step from the condition described by Surface in a Polyclad to that which I have observed in the Rhabdocoele, Paravortex. Here two entoderm elements—the large macromeres of text figure 14—give rise by division to the primary and secondary entoderm cells. The former, since they contain no yolk of their own, absorb it from the vitellarial substance. Like the upper yolk cells in Planocera, their nuclei degenerate and their membranes break down. But this latter

step occurs only after they have been ingested by the secondary entoderm cells, which thus resemble in behavior the cells of the alimentary canal in Planocera.

Hallez states that in P. cardii no membrane is formed over the surface of the yolk mass by a part of the vitellarial cells. P. gemellipara resembles P. cardii in this respect; no shell-membrane arises. But Hallez thought he found a similarity between the behavior of the yolk-cell nuclei of Paravortex and Mesostomum in that certain of these nuclei in the former played an important part in the differentiation of the definitive ectoderm and primary intestine; according to Bresslau, in Mesostomum ehrenbergi, the peripheral yolk cells form a shell membrane over the surface of the remaining yolk. In Paravortex gemellipara, however, every cell which enters into the structure of any organ is a derivative of the egg. The vitellarial yolk-cell nuclei and membranes disintegrate, leaving only the yolk as food; the nuclei never contribute to the formation of living tissue.

Of the mitochondrial mass little remains to be said. Its history from the first appearance in the young oocyte was traced until it finally disappeared in the entoderm of the young worm. The suggestion was tentatively made that the body might be or might produce an enzyme which has to do with the transformation of the yolk. But a similar transformation took place in the ectoderm, a tissue which received none of the mitochondrial mass. It therefore appears that the literature concerning the 'Dotterkern' has received another contribution, but without further explanation of its action. Truly suggestive, however, is the behavior of the body.

VI. SUMMARY

1. The origin of two or more embryos found in the capsules of Paravortex gemellipara is similar to their origin in other capsule-forming Rhabdocoeles; that is, by the enclosure of two or more eggs and about one hundred yolk cells in a shell secreted by the shell glands.

2. The mitochondrial mass (yolk-nucleus) was traced from its origin in the young oocyte through cleavage to its final resting

place in the several primary entoderm cells. Its function probably has to do with the absorption and digestion by these cells of the vitelline yolk. The behavior of the mitochondrial mass is different from that observed in other animals in that, whereas in most forms it either disappears in the egg stage or, if remaining through cleavage, is restricted to one cell, in P. gemellipara several cells ultimately receive a portion of the original substance.

3. Three germinal regions are recognizable in the blastula, the mes-ectoderm, primary entoderm and secondary entoderm. These germinal regions do not seem to have been previously recorded in Turbellaria.

4. The vitellarial yolk-cell membranes and nuclei degenerate after deposition in the capsule; no temporary epithelium is formed by them outside the yolk mass.

5. The primary entoderm cells which lie as a cap at the posterior end of the embryo, absorb a large part of the vitellarial yolk. When replete their nuclei immediately degenerate.

6. These cells are then grasped by the amoeboid secondary entoderm cells. After gastrulation the latter completely engulf and digest the former.

7. During gastrulation the ectoderm cells absorb the free vitellarial yolk left over after the action of the primary entoderm.

8. The development of the eyes embraces two phases. First the accessory pigment cell arises as a large clear vesicle very early in the differentiation of the embryo, and becomes gradually filled with melanin granules which crowd the nucleus to one side. Secondly the essential organ, the sight cell, develops externally to the pigment cell, into an invagination of which it pushes, and sends a nerve fiber backward into the anterior horn of the brain.

9. The sub-dermal muscle fibers develop in this Rhabdocoele, as in Nemerteans, by the differentiation of mes-ectodermic cells from which long slender fibers grow outward beneath the external epithelium.

10. The intestine consists of large distinct cells whose free ends extend into the lumen. It arises after birth by the inter-

calation between the yolk-laden primary entoderm cells of new
cells derived from the secondary entodermic elements which
during gastrulation remain in the region just posterior to the
oesophagus.

11. The approximate time of appearance was noted for the
subdermal glands and those of the alimentary tract; they are
not present in the young worm at the time it leaves the mother,
but have already developed in free-living specimens which have
reached a length of 0.30 mm.

12. The cephalic glands appear in the embryo while it still
lies in the capsule. There are three single-celled glands which
develop in the mesenchyme between and anterior to the eyes.
By the time the young worm is ready to leave the mother these
glands have each become connected with the exterior through
a fine process which penetrates the anterior body covering.

13. During very early embryonic life the vitellogenous cells
migrate backward from the region of the gonads—just posterior
to the pharynx. Most of them become grouped in the pos-
terior end of the body, but a number come to rest at various
points beneath the ectoderm in the posterior two thirds of the
body. The vitellaria develop first from the large mass of cells
between the intestine and the body wall and push gradually
forward till they effect a union with the posterior end of the
antrum. Meanwhile the isolated cells divide to form groups
which soon anastomose with the main portions of the glands.

The testis and ovary of each side of the body become differ-
entiated from a single gonad, the former arising from the an-
terior and the latter from the posterior end. In a 0.30 mm.
embryo the two glands have become separated. Meanwhile
a large group of cells has accumulated in the mid-ventral line
between intestine and body wall. The anterior portion of this
mass proliferates cells which surround the testis with a flat
external epithelium and also form the vasa deferentia. The
seminal vesicle arises in the next posterior portion of the mass
by a rearrangement of cells about a lumen. It is composed
of two layers of epithelia. At the same time the atrium com-
mune develops in a similar manner. At this stage the antrum

femininum and oviducts exist only potentially in the undifferentiated mass posterior to the atrium.

The shell glands develop below the antral mass before the lumen of the latter appears. At this stage the testis has become functional; one can easily distinguish spermatogonia at the anterior end, the primary and secondary spermatocytes, spermatids and mature spermatozoa. Many of the latter now appear in the thick-walled seminal vesicle. The genital pore does not open until later.

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1   Outline from life of P. gemellipara somewhat flattened under a cover slip.   Several stages of embryonic development shown within capsules   × 64.

2   Three animals natural size and five others × 5 in characteristic shapes and poses.

3   The entire animal viewed from the left side as a semi-transparent object. To show particularly the position of the reproductive organs and their relation to other organs.   × 88.

4   Frontal section through the upper end of the antrum femininum, the ends of the vitellaria and the most mature oöcytes of each ovary.   × 200.

5   A spermatozoon.   × 606.

6   Youngest third of the ovary to show the oögonia, development of the nucleolus, and particularly the development of the mitochondrial mass.   × 606.

7   Portion of an oöcyte to show the structure of the cytoplasm and mitochondrial mass.   × 606.

ant.♂, antrum masculinum
ant., antrum femininum
at., atrium commune
br., brain
cap., capsule
cyt., cytoplasm
e., eye
gl.c., cephalic glands
gl.sh., shell glands
g.p., genital pore
int., intestine
m., mouth
mes., mesenchyme
mit., mitochondrial mass

nat.s., natural size
n.op., optic nerve
nuc., nucleus
nucl., nucleolus
o., ovary
ooc., oocyte
oog., oogonia
ph., pharynx musculature
s.v., vesiculum seminalis
t., testis
v.d., vas deferens
vest., vestibule
vit., vitellarium
y.c., yolk cell
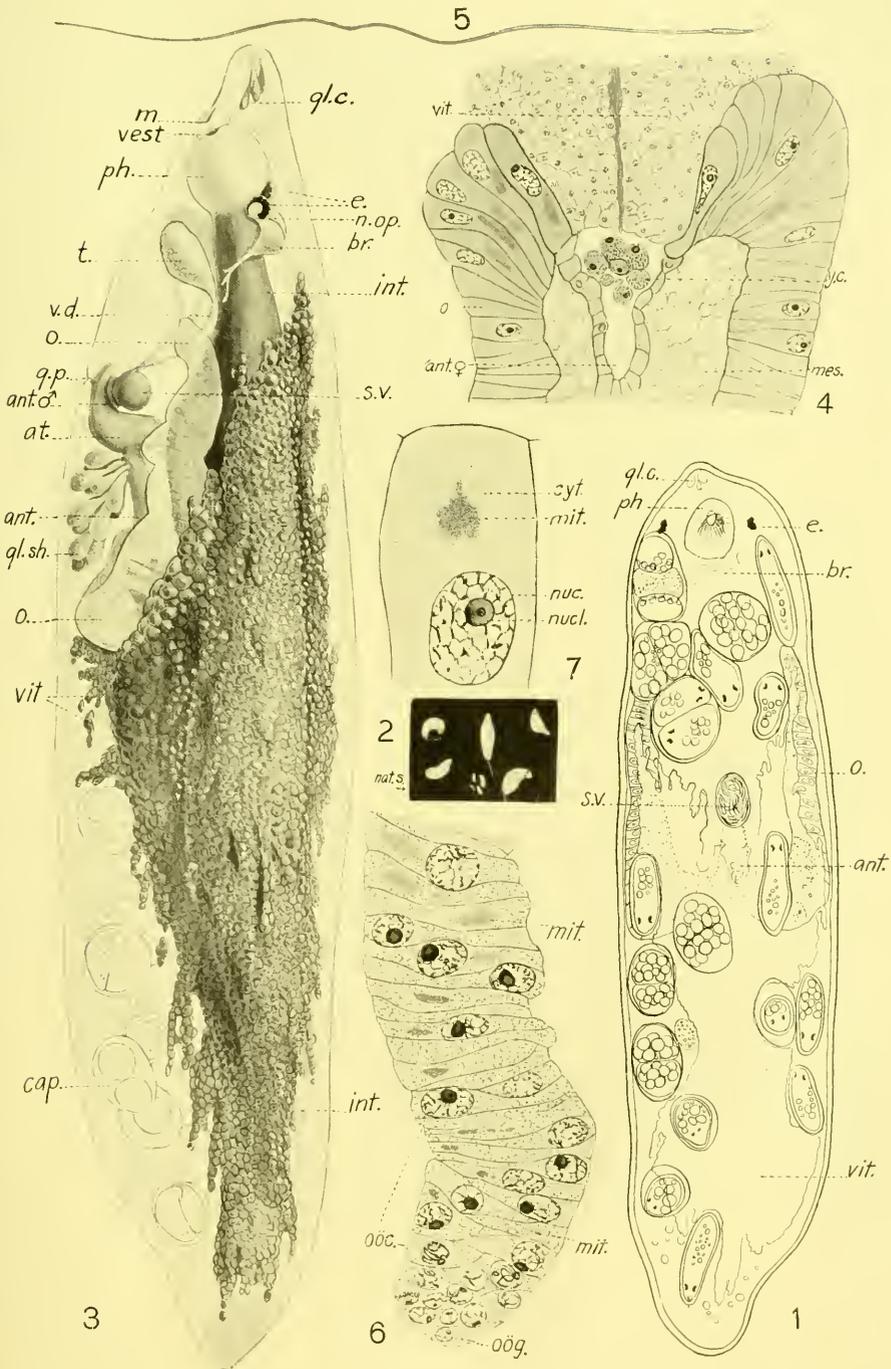
EXPLANATION OF FIGURES

8 Frontal section through the posterior end of the ovary as it curves inward toward the oviduct. Note the definite membranes about the ova. × 270.

9 Oblique sagittal section showing the crucial stage in the formation of the capsule. Two ova are being surrounded together with yolk cells by the secretion of the shell glands. Numerous spermatozoa present about the eggs, and a mass of them in the antrum. × 270.

ant., antrum femininum
at., atrium commune
int., intestine
mit., mitochondrial mass
o., ovary
sh.gl., shell glands

sh.sec., secretion of shell glands
sp., spermatozoon
s.v., vesiculum seminalis
vit., vitellarium
y., yolk

8

9
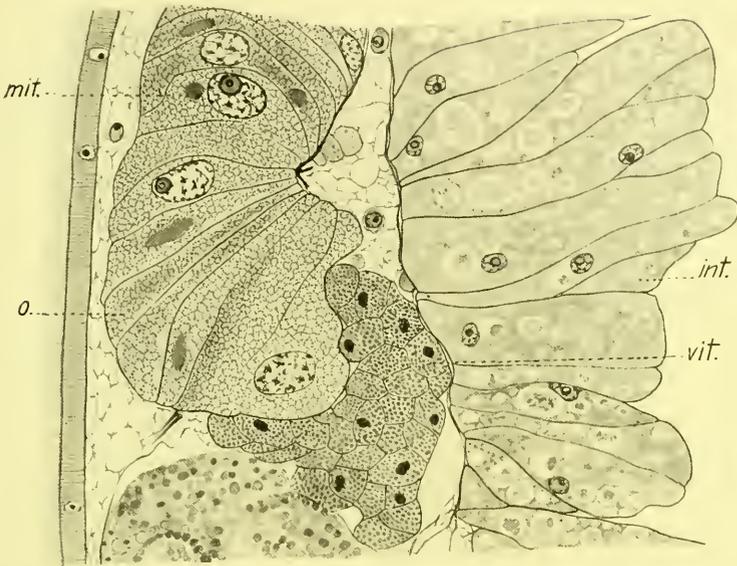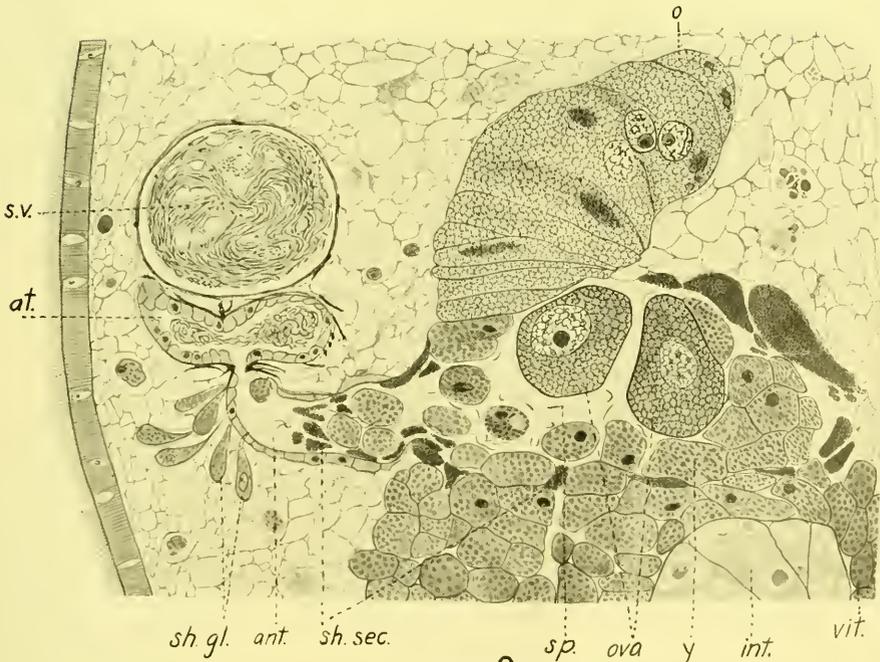
EXPLANATION OF FIGURES

10   Section of capsule showing first polar body at the left of the egg, and the male pronucleus inside.   Several degenerating yolk cell nuclei and one degenerating spermatozoon in the yolk.   × 730.

11   Another section of the same capsule in which the yolk cell membranes have not yet disintegrated.   × 730.

12   Drawing from life of a capsule freed from the mother by pressure, showing natural condition of yolk and mitochondrial mass, one egg and one embryo. × 520.

13   Two eggs of one capsule showing progressive steps in the cutting off of the first micromere.   × 730.

14   Section through an embryo of about 72 cells.   Three germinal regions are indicated, the primary entoderm (4 cells showing) at the left, mes-ectoderm at the right and secondary entoderm lying between.   Yolk cell membranes and nuclei have entirely disintegrated.   × 730.

15   Drawing from life of an embryo at the same stage as that in the preceding figure.   × 730.

cap., capsule

cent., centrosome

d.sp., degenerating sperm

d.y.n., degenerating yolk cell nucleus

ent.1, primary entoderm

ent.2, secondary entoderm

inf., infiltration

mic.1, first micromere

mit., mitochondrial mass

p.b.1, first polar body

y., yolk

y.c., yolk cell
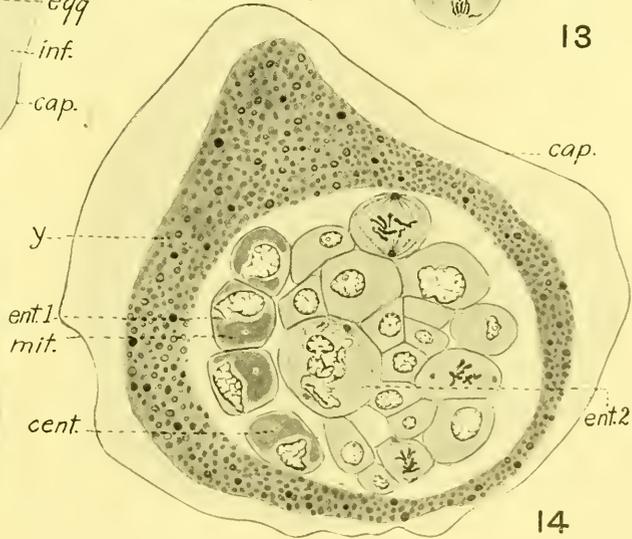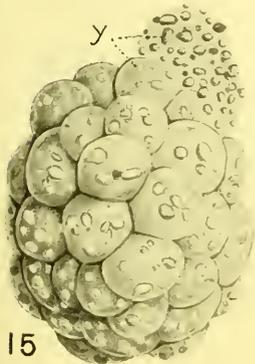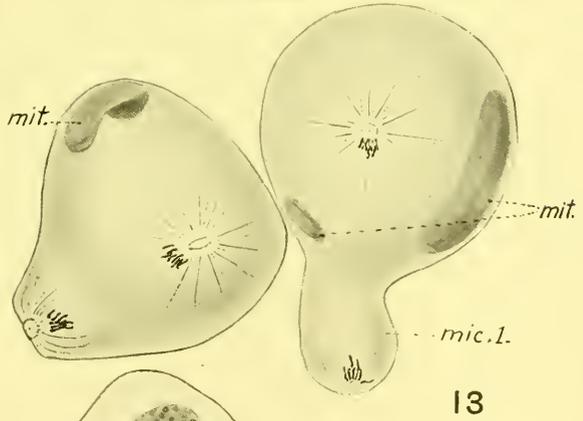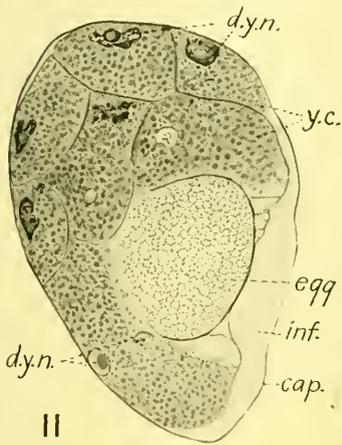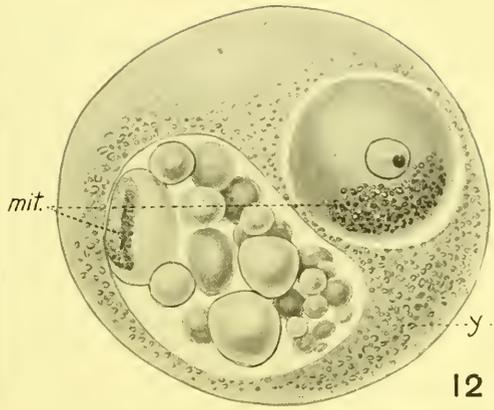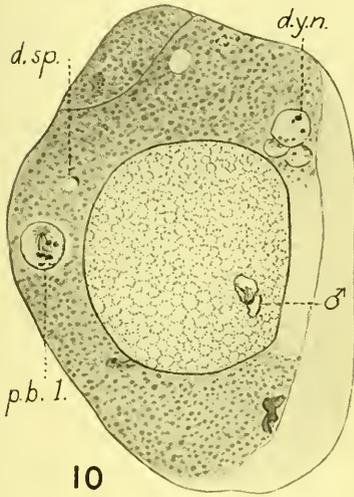
♂,   male pronucleus

16 Section through an embryo to show clearly the three germinal regions, mes-ectoderm, primary and secondary entoderm. × 730.

17 Section through a capsule of which the two embryos have begun to absorb yolk. This process is carried on by the secondary entoderm cells; each shows the mitochondrial mass and several yolk granules. × 730.

18 Section through part of a capsule in which the secondary entoderm cells have wandered to the posterior side of the primary entoderm. Most of the yolk has been absorbed by the entoderm. × 730.

19 Cell showing probable method of division of the mitochondrial mass. × 520.

20. Mitochondrial mass after its first division. Centrosome inside at the left, outside at the right. × 520.

cent., centrosome
d.y.n., degenerating yolk cell nucleus
ect., ectoderm
ent.1., primary entoderm

ent.2, secondary entoderm
mes-ect., mes-ectoderm
mit., mitochondrial mass
y., yolk

547
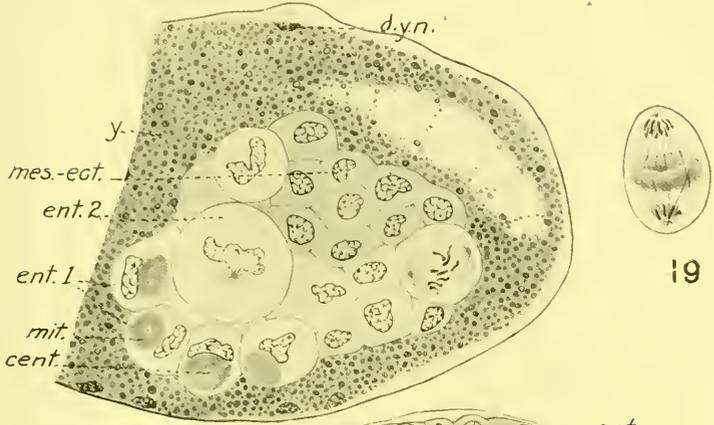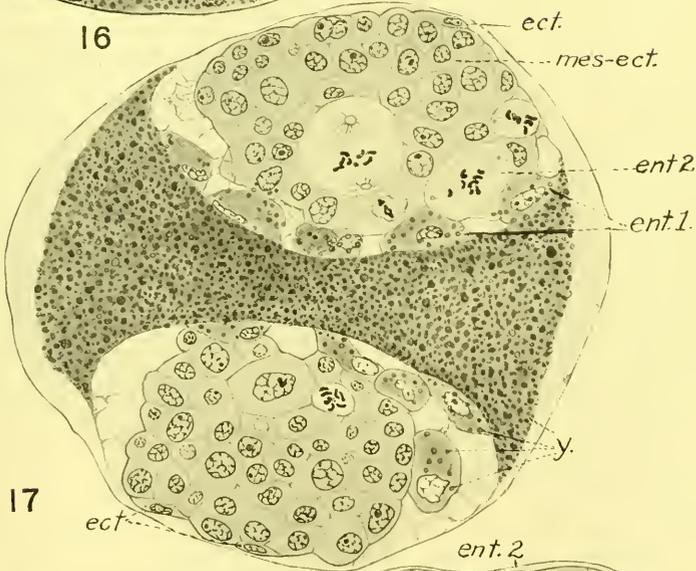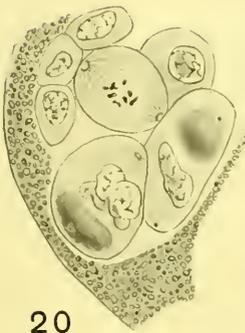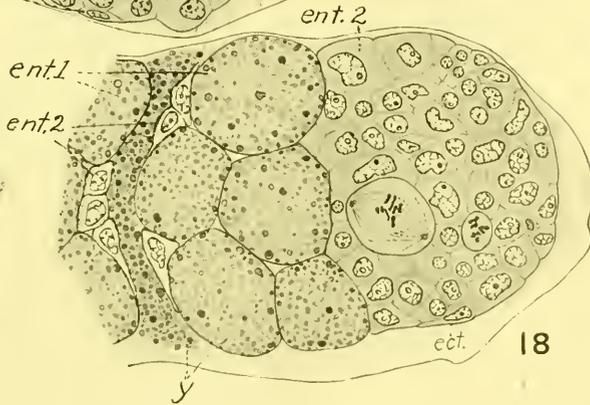
PLATE 5

21 Section through two embryos of a capsule abundantly supplied with yolk. Nuclei of the primary entoderm cells are undergoing degeneration while these cells are being engulfed by those of the secondary entoderm. At *ect.* the mes-ectodermic cells are becoming flattened to form the ectoderm. × 730.

22 Thick section of capsule in which the upper embryo is in the process of gastrulation. The ectoderm cells have nearly closed over the entoderm. The primary entoderm cells have been grasped by those of the secondary entoderm. At the right three ectoderm cells are absorbing free yolk left in the capsule after the entoderm had become replete. Ectoderm at the left contains yolk which has already been partially transformed. × 730.

23 Oblique frontal section of an embryo just after gastrulation has been completed. Same features as in preceding figure. The ectodermic yolk beginning to be transformed (cf. the two cells at the top). The cells from which the intestine is mainly to be derived are shown (*ent. 2*) lying between the yolk-laden cells and the undifferentiated anterior cell mass. On the lower right several large nuclei with their cytoplasm are to be added to the ectoderm. × 730.

24 Oblique sagittal section of a slightly later stage in which the mesodermic cells have pushed backward between the posterior body wall and entoderm. Eye pigment cell just differentiated. × 730.

cap., capsule
ect., ectoderm
ent.1, primary entoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
mes., mesenchyme

mes-ect., mes-ectoderm
ph.ep.int., internal pharyngeal epithelium
t.y., transformed yolk
y., yolk
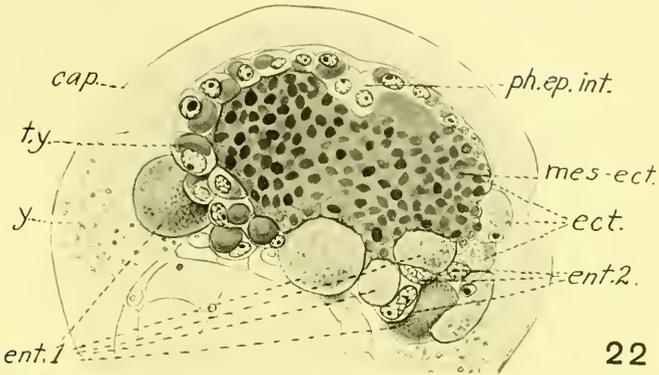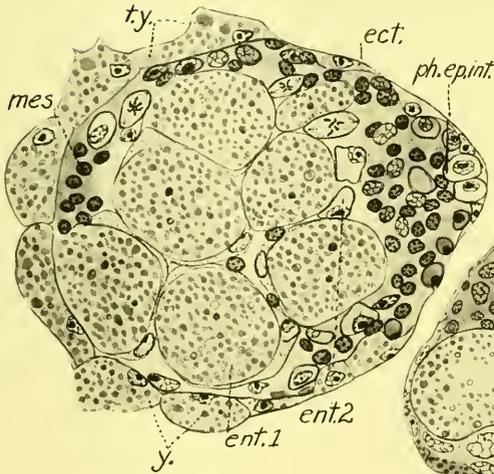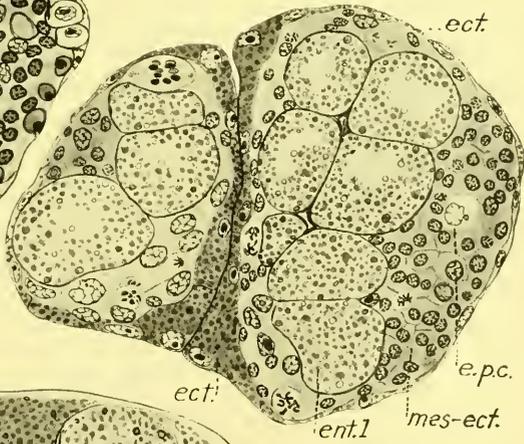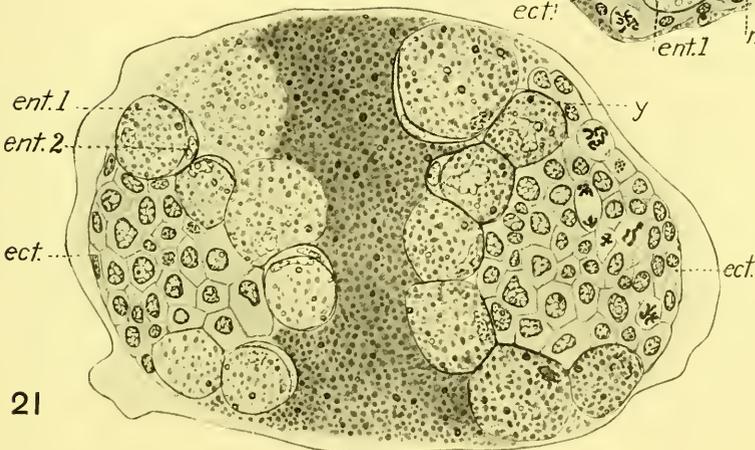
25   Drawing from life of an embryo at the stage seen in the preceding section (fig. 24).   The globular nature of the free yolk is well shown.   Water had infiltrated under observation between capsular membrane and yolk.   Stained intra vitam with neutral red.   × 730.

26   Frontal section of an embryo at same stage as that in preceding figure. Same features as in the latter and also the secondary entoderm cells (*ent. 2*). × 520.

27   Transverse section through pharynx and most anterior entoderm cells. Internal pharyngeal epithelium clearly illustrated in section, and the cells which are to form the pharynx musculature are grouped around this epithelial rod. × 520.

28   Frontal section of anterior end of an embryo to illustrate position of the two eye pigment cells.   × 1016.

29   Oblique transverse section through brain and posterior nerve trunks. × 730.

30   Series of drawings to show one type according to which the pigment granules of the eye appear one after another.   × 530.

31   A second series to show another type of melanin deposition whereby several tiny spherules appear together and enlarge gradually.   × 530.

32   Eye of an older embryo which had escaped from its capsule.   Pigment cell has become invaginated by pressure of the ganglion cell (*g.c.*) from which nerve fibers extend into the brain.   × 730.

33   Adult right eye seen from above showing the sight cell at the end of the optic nerve.   × 730.

br., brain
ect., ectoderm
ent.1, primary entoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
esoph., esophagus
g.c., ganglion cells
inf., infiltration
m.emb., embryonic muscle cell
mes., mesenchyme
mes-ect., mes-ectoderm

n., nucleus
n.op., optic nerve
n.p., posterior nerve trunk
pct.s., punktsubstance
ph., pharynx musculature
ph.ep.int., internal pharyngeal epithelium
t.y., transformed yolk
vest.ep., epithelium of vestibule
y., yolk
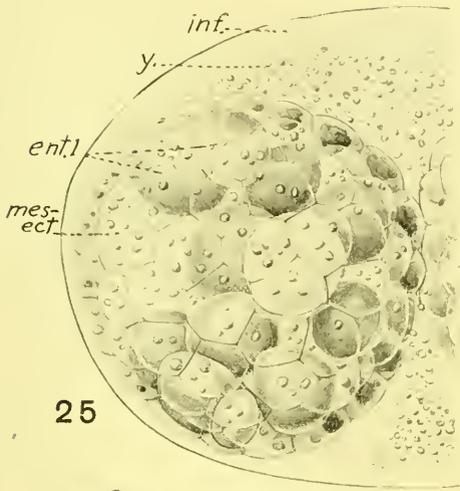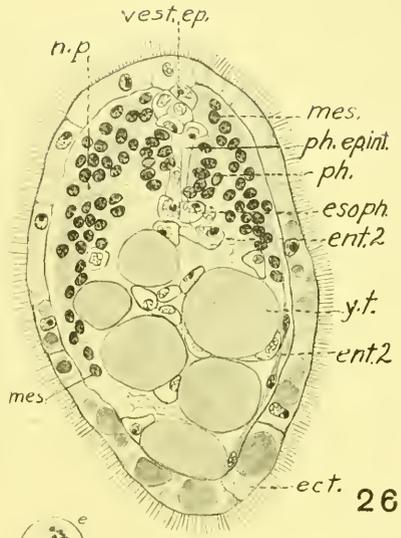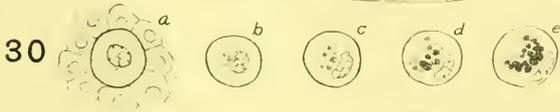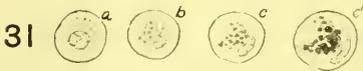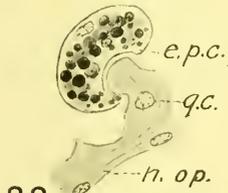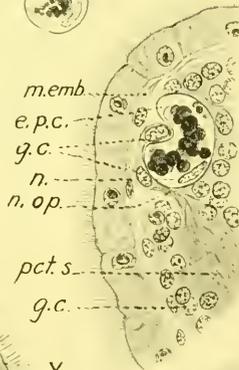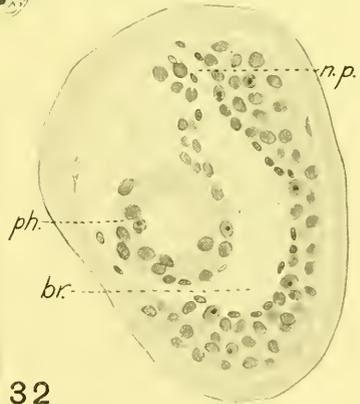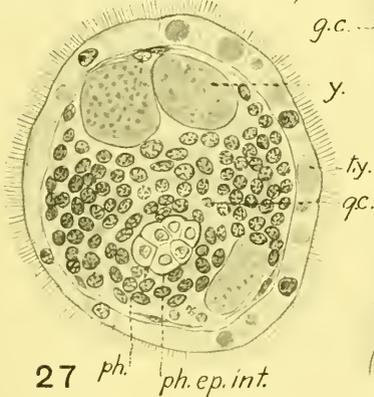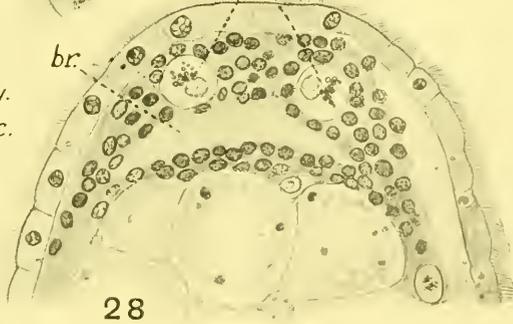
PLATE 7

34 Oblique sagittal section of an embryo somewhat more advanced than that in figure 33. Digestion of the yolk has begun; clear masses of transformed yolk occupy the center of each entoderm cell, while in the ectoderm the yolk has formed several irregular masses in each cell. The anterior ectoderm has become vacuolated. Between ectoderm and entoderm muscle cells are forming from mesodermic elements. Brain has differentiated and the internal epithelial rod has grown inward through the indistinct mass of pharyngeal muscle cells. Position of the mouth indicated at m. $\times$ 530.

35 Oblique sagittal section showing brain, eye pigment cell, entoderm and muscles. $\times$ 530.

36 Transverse section of same embryo as that shown in figure 27, but farther posteriorly, passing through the cells which later form the esophagus. $\times$ 530.

37 Oblique sagittal section of an older stage than that shown in figure 34. Entodermic yolk completely transformed (does not stain with iron haemotoxylin) while that in the ectoderm exists as large spheres. Pharynx distinct and early vitellogenous cells (vit c.) differentiated. Cilia have developed. $\times$ 730.

38 Transverse section of pharynx to show its musculature, and internal and external epithelia. $\times$ 730.

39 Tangential section through body wall of an embryo which had become freed into the mesenchyme of the mother. Muscle cell (m. emb.) from which extend fine muscle fibrils; the latter become the definitive sub-dermal fibers. $\times$ 1016.

40 Portion of a transverse section of the dorsal body wall showing outer circular and inner longitudinal sub-dermal muscle fibers. $\times$ 1016.

41 Adult sub-dermal muscle fibers. $\times$ 1016.

ect., ectoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
esoph., esophagus
g.c., ganglion cells
gon., gonad
m., mouth
m.emb., embryonic muscle cell
m.f.c., circular muscle fibers
m.f.l., longitudinal muscle fibers

pct.s., punktsubstance
ph., pharynx musculature
ph.ep.int., internal pharyngeal epithelium
ph.ep.ext., external pharyngeal epithelium
t.y., transformed yolk
vit.c., vitellogenous cells
y., yolk
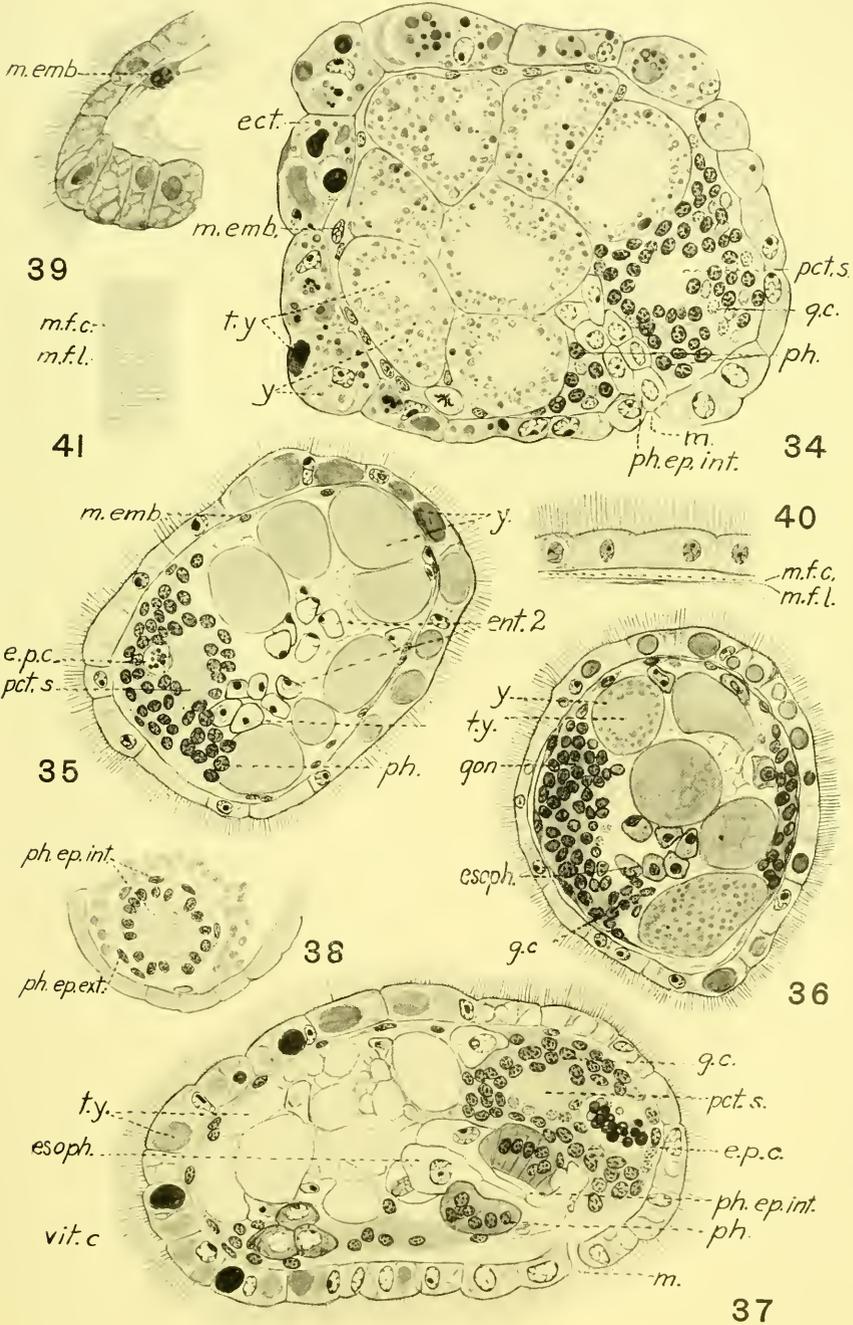
42 Frontal section of an embryo somewhat younger than that in figure 37. The yolk masses are seen to be lying inside the secondary entoderm cells, having lost the membranes of the primary entoderm. Mitochondrial mass still visible with its centrosome. Yolk in the upper cell (*t.y.*) has been transformed to a fluid mass. $\times$ 730.

43 Sagittal section through an embryo which had not yet left the capsule. Drawn to show the vitellogenous cells migrating posteriorly. $\times$ 730.

44 Oblique transverse section through an embryo still in its capsule. Several large vitellogenous cells appear between the entoderm and sub-dermal muscle layer. Above are shown for comparison several cells at the tip of one of the mother's vitellarial strands. $\times$ 730.

45 Anterior end of an embryo in which the cephalic glands are differentiating. Embryo still in capsule. $\times$ 730.

46 Cephalic glands at a later stage showing outlet to the exterior. Embryo free in the mother's mesenchyme. $\times$ 730.

47 Oblique frontal section of a young worm 0.30 mm. long found in sea water in which mussels had been washed. Relation of young testis and ovary is shown, as well as the fact that the vitellaria develop from the posterior mass of vitellogenous cells (figs. 43, 44). Cellular nature of the intestine is apparent. Subdermal and intestinal glands, and the structure of the mesenchyme are indicated. $\times$ 450.

48 Seminal vesicle of living adult containing several spermatids. $\times$ 808.

br., brain
e., eye
ent.1, primary entoderm
ent.2, secondary entoderm
gl.c., cephalic glands
gl.int., intestinal glands
gl.sub., subdermal glands
int., intestine
m.emb., embryonic muscle cell
mes., mesenchyme
mit., mitochondrial mass

o., ovary
ph., pharynx musculature
sp., spermatozoon
spt., spermatid
s.v., vesiculum seminalis
t., testis
vit., vitellarium
vit.c., vitellogenous cells
x., vitellogenous cells in mitosis
y., yolk
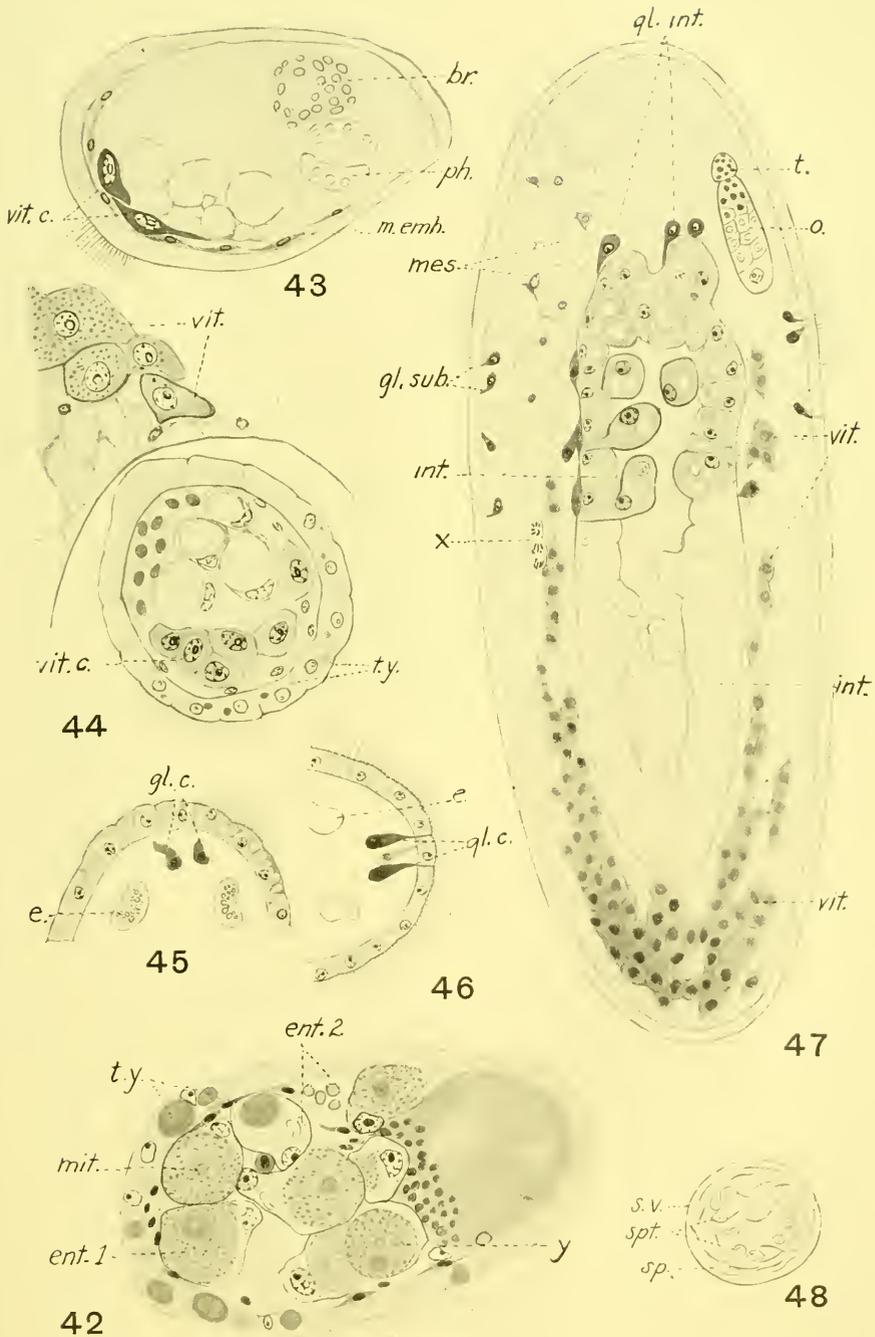
49 Lateral view of anterior end of an embryo which had entered the mother's intestine. Drawn to show the gonads with cells in mitosis. $\times$ 730.

50 Sagittal section through the reproductive organs showing the undifferentiated cell mass which is to give rise to the atrium, antrum femininum and oviduct; also the shell glands (*gl.sh.*). $\times$ 400.

51 Sagittal section showing later stage in the development of the sexual organs. Testis has become functional showing spermatogonia, primary and secondary spermatocytes and mature spermatozoa. A number of the latter have already reached the seminal vesicle. Not yet has a lumen been formed in the antral region. $\times$ 400.

52 Reconstruction from sections of the reproductive organs of a 0.60 mm. worm which had been liberated from the mother for some time. The point of view is one looking posteriorly from a point just in front of the testes. $\times$ 270.

53 Oblique frontal section of an abnormal embryo in which the primary entoderm cells, having failed to absorb yolk according to their function, still remain in the center of the unusually small posterior half. The mesenchymatic cells have proliferated as in normal embryos and appear in several rows. $\times$ 530.

ant., antrum femininum
at., atrium commune
br., brain
ent.1, primary entoderm
ep.t., epithelium of testis
esoph., esophagus
gl.sh., shell glands
gon., gonad
g.p., genital pore
o., ovary

o.d., oviduct
ph., pharynx musculature
sp., spermatozoon
spc.1, primary spermatocyte
spc.2, secondary spermatocyte
spg., spermatogonia
s.v., vesiculum seminalis
t., testis
v.d., vas deferens
vit., vitellarium
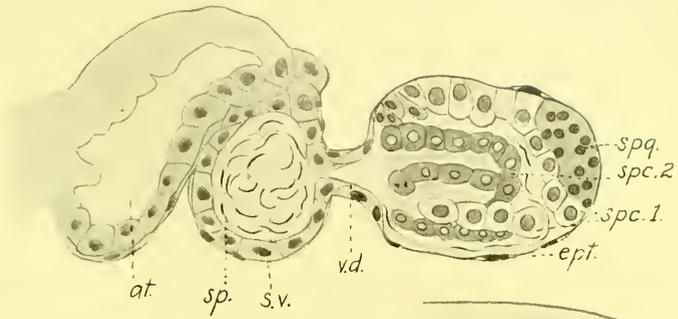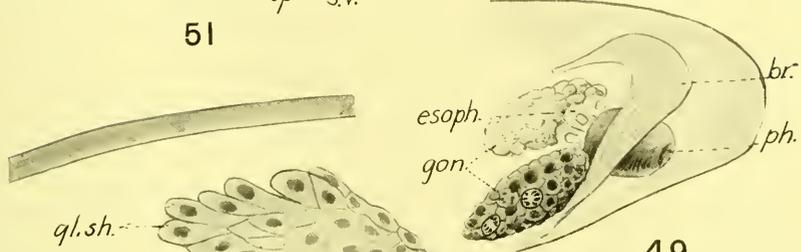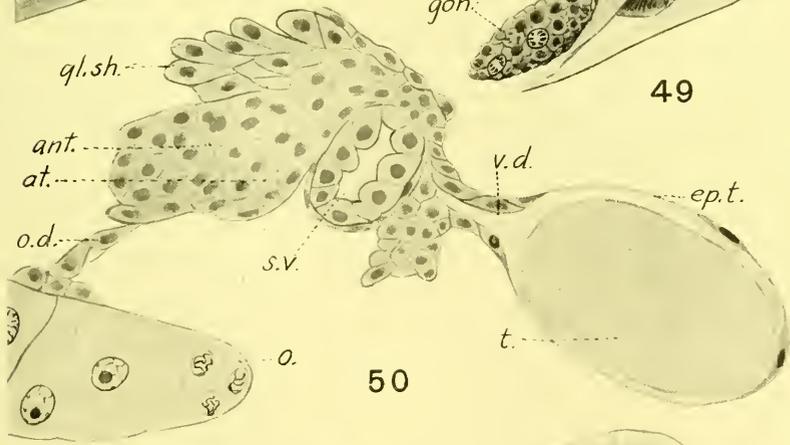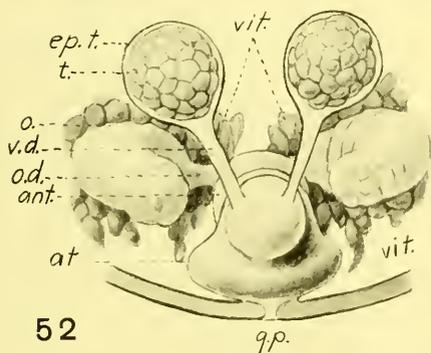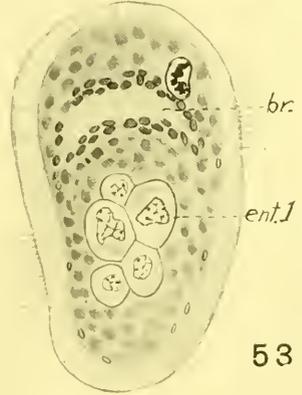
STUDIES ON CELL DIVISION AND CELL DIFFERENTIATION

I. DEVELOPMENT OF THE CELL ORGANS DURING THE FIRST CLEAVAGE OF THE SEA-URCHIN EGG

VERA DANCHAKOFF

THIRTY-ONE FIGURES (FIVE PLATES)

1. INTRODUCTION

The morphological changes of the living substance, exhibited in our preparations, refer only to certain stages of the complex physico-chemical process which is involved in the history of the cell. Unfortunately, isolated phases of cell development often become the subject of independent studies and the inseverable connection between the different stages of cell development became gradually lost.

The paricular interest attaching to the nucleus began with its discovery. Observations on its physiological activity seemed to indicate it as the regulative center of the metabolic processes in the cell. The discovery of chromatin in the nucleus and of the differentiation of chromosomes during cell division attracted the attention of investigators to the specific rôle of the nucleus as bearer of hereditary qualities. And both functions relating to the nucleus, different as they may be, seemed to find a material basis in the chromatin, which was admitted to be localized not only within the nucleus, but also in the cytoplasm.

Investigators have repeatedly described the presence of chromatin in the cytoplasm of both protozoa and metazoa. Although the nucleus was invariably considered as the permanent primary source of the extranuclear chromatin, the interpretation of the rôle assigned to the extranuclear chromatin has widely differed. Hertwig's followers assume a permanent presence of chromatin in the cytoplasm. Hertwig himself gave to the extra-

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nuclear chromatin the name of somatochromatin; the chromatin of the nucleus became known as idiochromatin. According to Hertwig, the somatochromatin is activated iodiochromatin, the potency to activity being in idiochromatin in a latent stage. Subjected to certain conditions, idiochromatin may be transformed into somatochromatin. The somatochromatin has a trophic influence upon the cell activity, and is continually transferred from the nucleus to the cytoplasm. Since the extranuclear chromatin is subjected in the cytoplasm to a gradual destruction, the permanent emission of chromatin by the nucleus would prevent the hypertrophy of the nucleus.

Rückert also considers the somatochromatin or trophochromatin, as a permanent element in the cytoplasm, but draws a strong distinction between the somato- and reproductive chromatin. The somatochromatin has trophic regulative function, the reproductive chromatin is connected with transmission of hereditary qualities.

Schaudinn and Goldschmidt extend the distinction between the two kinds of chromatin still farther. According to them, the nuclei of every animal cell are duplex in their organization and contain chromatin of reproductive and of merely somatic function. The chromidial apparatus of the cell, by which name the extranuclear chromatin is termed, arises from the somatic nuclear substance.

A somewhat different opinion is expressed by Schaxel. This author comes to a conlcusion, that the extranuclear chromatin, present in large masses in the egg and in the blastomeres of sea-urchins is emitted by the nucleus at definite stages of cleavage. Schaxel does not admit continuous emission of chromatin by the nucleus. The chromatin particles in the cytoplasm, according to him, are 'Kinetochromidien' and contain Weismann's determining biophores. During cleavage they undergo progressive exhaustion and are not capable of synthesis in the cytoplasm.

Thus the rôle of chromatin in the cytoplasm has been differently interpreted. It was considered by some investigators as a substance, from which the nucleus endeavors to free itself, and which finally disintegrates in the cytoplasm. Again others

described the presence of chromatin in the cytoplasm as a periodic one: exerting a definite function, the extranuclear chromatin appears only during brief but definite periods in the development of the cell. Finally the extranuclear chromatin has been considered as a permanent element in the cytoplasm with a particular trophic function opposed to that of the real nuclear reproductive chromatin. The extranuclear chromatin has been invariably derived from the nucleus. The independence of the nucleus and the cytosome seemed, however, to be established so firmly, that the observations upon the chromidial formations in the cytoplasm were received with great scepticism.

In recent time there were discovered in the cytoplasm structures analogous to the chromidia, but, in their origin, quite independent of the nucleus. Meves and Duesberg called them mitochondria or plastosomes. They attributed to these structures an importance similar to that of the nucleus, since both, in their opinion, were bearers of hereditary qualities. Just as a nucleus has always for its genesis a division of a mother-nucleus, so have the plastosomes for their origin a division of a mother-plastosome. They are in other terms, self-propagating units. The discovery of the plastosomes added a new cell-organ to an extensive series already known.

The cytoplasm, the nucleus, the somato- and idiochromatin, the mitochondria, and finally the centrosome, all these cell-parts were regarded as independent in their existence and genesis, and capable of self-multiplication. The cell seemed to become a series of coexisting, independent, self-propagating units. The lack of firmly established notions either of the nucleus, its chromatin and nucleolus, or of the centrosphere, not to mention the more recently discovered plastosomes and chromidia, is greatly due to isolated studies of artificially separated stages of cell development.

Often, as for example in the question of the centrosome, opinions of prominent biologists seemed to diverge widely. For Boveri the centrosome was an organ, present in all animal cells, an organ, which was transmitted from one cell generation to another by multiplication. For Hertwig the centrosome was a temporary

organ, which disappeared after it had fulfilled its function during cell division.

Such controversies seemed to depend largely upon the general view, that the cell organs are independent formations, equal in all living cells. The principle of evolution, either phylogenetic or ontogenetic, was not applied to the organs of the cell. Any observation concerning the cell organs of different animals and of different stages of development had to strictly correspond to definite formula. Data, different only because they belonged to various periods and different classes of animals, were regarded as wrong and were rejected as not fitting into the artificially created rigid limits of fixed theories. The principle of evolution of different classes of animals and the principle of evolution of different stages of organ development, both aspects of the same law, do not constitute the whole content of the law. The principle of evolution appears as a most general quality of living matter, and no tissue, no cell, no cell organ, can escape its opertion. The different numbers in chromosome complexes of different animals and the different aspects of mitotic figures in different tissues of the same animal may serve as an example.

In my former studies on the development of blood in birds and reptiles, the ontogenetic development of a definite kind of blood cells and their organs was repeatedly taken into consideration. The small size of the blood cells, however, prevented a more detailed study of the ontogenetic development of cell organs. The study of the beautiful, transparent eggs of Strongylocentrotus lividus seemed to offer an opportunity of verifying some conclusions drawn from the study of differentiation of blood cells. Some new facts observed in the development of the eggs may help to correlate our conception of different morphological structures in the cell.

2. MATERIAL AND METHODS

The material for the present study consists of a large series of sea-urchin eggs, fixed before fertilization and at different stages of their development after fertilization. The material has been collected at the French biological station at Roscoff, whose kind hospitality I highly appreciate.

Observations made in previous studies on hematopoiesis invariably indicated the existence of wonderful gradation in normal development and differentiation of cells, which seemed to offer a great hindrance for a detailed study of reciprocal relations of cell organs. Since changes in normal cell development could be incited by experiment, recourse to experimental method was indicated.

It is known, that the result of artificial stimulation of mature sea urchin eggs by different chemical substances is a parthenogenetic development of the egg. Besides the production of normal larvae, many eggs develop under artificial stimulation in a pathological way. A thorough study of parthenogenetic development of the egg as well as a study of those eggs, of which the development exhibited marked differences from the normal conditions, might have thrown light upon the correlation of different cell organs. Therefore, besides series of normally fertilized eggs, other series of eggs developed by artificial parthenogenesis were collected. The artificial parthenogenesis was produced by the method of Professor Delage and the experiments were carried out by Dr. de Beauchamp, the assistant of Professor Delage, to whom it gives me much pleasure to express my thanks.

Among different fixatives Flemming and Zenker-Formol fluids seemed to be most appropriate. As far as comparison of fixed preparations with living material allows to judge, these fixatives preserve all structures of the cytosome. Since both fixatives gave similar results, I used chiefly Zenker-Formol, because of the easier and better staining results following its use. The eggs were imbedded in paraffin, while care was taken not to exceed 40 C. Different staining methods were applied, preferably eosin-azur, saffranin and lichtgrün, and iron-haematoxylin.

3. STRUCTURE OF THE EGG BEFORE AND AFTER MATURATION

A cursory glance at figures 1 and 2, drawn from eggs before and after maturation, may show the enormous changes, which occur in the egg during maturation. Lack of adequate material did not allow me to follow gradually these changes, therefore

only description of the structure of the eggs before and after fertilization will be given with rerefences to the limited data found in the literature, which attempt to explain this marked difference in the condition of the eggs during these stages.

The most striking feature of a ripe but immature egg (fig. 1) is the presence of a very large nucleus. This nucleus contains a well developed large spherical nucleolus, which in different eggs may exhibit various aspects. This concerns chiefly its staining reaction, for sometimes it appears very homogeneous and intensely basophilic; at other times it stains very lightly not only with the azur but even with the iron-haematoxylin method. Its substance appears highly refractive, what enables its easy identification in living or unstained preparations. Some of the nucleoli, which gradually lose their basophilic reaction, manifest a striking vacuolisation, the vacuoles being filled by a less basophilic substance.

Besides the nucleolus, the nucleus contains a finely granular, intensely staining mass, together with a few thin twisted filaments, sometimes strongly basophilic, at other times hardly recognizable. Do these filaments represent chromosomes? It is difficult to determine. In the granular mass of the nucleus some larger particles of intensely basophilic substance can also be observed.

The cytoplasm of an immature egg is not homogeneous. In many places it appears denser and seems to contain peculiar formations, not always very distinctly separated from the adjacent cytoplasm. They appear as strings and larger ribbons or broken masses (fig. 1), and evidently correspond to the large mitochondria of insect spermatogonia, which were kindly demonstrated to me by Prof. McClung and Miss Brockett.

At this stage the egg does not present any other definite structures—there is no trace of a centresome, centrioles, or chromidia. However, the egg presents at its periphery two distinct layers, both separated from the rest of cytoplasm by more or less precise boundaries. These layers are not always easily discernible and are exempt of any definite morphological structure. The outer layer is very homogeneous, the inner layer is

slightly granular (fig. 1). Thus, the cytoplasm of a ripe but immature egg is differentiated in three layers, concentrically arranged around the nucleus.

The maturation of the egg leads to extensive changes both in the nucleus and in the cytoplasm. The most striking phenomenon is the change in the relation of the nucleus and the cytoplasm, this change occurring quite abruptly (fig. 2).

Hertwig, in his studies on 'Kernplasmarelation,' finds in the immature egg conditions of depression, similar to conditions of senescence in Actinospherium and Infusoria, in which cases the nucleus also acquires large dimensions. According to him, the reduction of the nucleus during maturation enables the further development of the egg cell. Minot and Conklin on the contrary attribute to the increase of nucleus a meaning of rejuvenescence and believe that senescence depends upon the increase of the cytoplasm and upon its differentiation.

These differences of opinion regarding the bearing of the Kernplasmarelation may be accounted for by studies of cellular processes, without due consideration of their mutual relation. Decrease or increase of the coefficient of the Kernplasmarelation may depend upon different causes and can have different meanings.

Conklin and Minot rightly consider the fall of the coefficient of the Kernplasmarelation as an indication of sensecence, but they refer to a fall of the coefficient, due to a collection of differentiation products in the cytoplasm. The fall of the coefficient during maturation of the egg is of different origin, therefore has a different meaning. Have the changes, which are observed during maturation, the character of differentiation processes? On the contrary, the structure of a mature egg becomes simpler. The morphological structures of the cell which characterize an immature egg, such as the nucleolus and the filaments in the nucleus, are no longer discernible in the mature egg. Even the three different layers of cytoplasm are reduced to two: a very thin peripheral layer, traversed by numerous parallel striae and the greater central mass of the cytoplasm. The egg now contains a very small nucleus. The greater part of the contents

of the nuclear vesicle of the immature egg has now become a part of the cytoplasm. The nucleus of a mature egg does not contain definite structures aside from occasional chromatic granules; its stroma is lightly acidophilic and only under the highest magnifications presents a lightly granular structure. The cytoplasm still contains more or less numerous mitochondria, which do not appear very distinct in my preparations.

But quite new morphological structures have appeared in the cytoplasm of the mature egg cell. These are somewhat large, spherical accumulations of a very intensely basophilic and refractory substance. They are very different in their size and number. There is, however, a somewhat definite relation between their number, size, and distribution; if they are small, they are numerous and more or less uniformly distributed through the cytoplasm; if they are larger, they are few and irregularly placed.

The spherical accumulations of the basophilic substance are chiefly found in the cytoplasm, yet sometimes one or two smaller accumulations of similar basophilic substances are observed within the nucleus. These usually appear in the form of small granules closely flattened against the nuclear membrane in the form of discs (fig. 2).

Since both in normal fertilization and in artificial parthenogenesis an important function seems to be assigned to the basophilic accumulations in the cytoplasm, it may be permitted to cite a paper of Schaxel, dealing with analogous structures. Studying the maturation processes in the sea urchin eggs, Schaxel considers the chromatin filaments in the nucleus as derived from the chromosomes. According to him, they condense later into nucleoli and finally join all together in one large nucelolus. This cell organ is, for Schaxel, a center of assimilation and emission of chromatin. The emission of chromatin by the nucleus is effected without conspicuous accumulations at the nucleus membrane, the chromatin being swiftly transported by currents. Lack of chromatin stagnation makes the process of emission of chromatin by the nucleus imperceptible and easily overlooked. The nucleolus appears after the emission of chromatin as an

achromatic body, is gradually vacuolized, and, after formation of chromosomes and breaking up of the germinal vesicle, is finally dissolved in the cytoplasm. The part of the chromatin, emitted by the nucleus, is distributed to the cytoplasm in the form of a diffuse substance. It is difficult to say, whether there is a full analogy between the diffuse chromatin found in the cytoplasm by Schaxel, and the precisely outlined accumulations of the basophilic substance in my preparations. If the origin of these substances seems to be similar, the fate of the basophilic accumulations in my preparations is different from that conceived by Schaxel for his Kinetochromatin. One will remember, that Schaxel admits a gradual exhaustion of the chromatin in the cytoplasm during cleavage.

One of my next problems will be the study of the origin of the basophilic chromatic accumulations in the cytoplasm. The present study is limited to the conditions exhibited by the mature egg. Here a small, spherical, more lightly stainable area of the protoplasm is differentiated into the nucleus. Its almost homogeneous structure is difficult to determine. The statement of Wilson, that "the terms nucleus and cell body should probably be regarded as only topographical expressions, denoting two differentiated areas in a common structural basis" could not be more exactly applied, than in the case of the mature egg of the sea urchin. While the small nucleus of the mature egg contains only occasionally one or two chromatin-granules (fig. 2), the cytoplasm abounds in accumulations of basophilic substance. This substance is stainable by every usual chromatin stain, exactly as are the small chromatin granules within the nucleus.

The occurrence of a peculiar chromatic substance in the cytoplasm in the early unicellular stage of a multicellular organism reminds of similar phenomena in the life of unicellular and lower multicellular organisms before and during their multiplication. Thus Bergh in Urastyla and Calkins in Tetraminus both describe in the cytoplasm the presence of numerous chromatin granules, and both find, that during the stage of cell division these chromatin granules accumulate in the center of the cell, and soon

form a single mass which elongates, assumes a fibrillar structure, and finally divides.

Scattered chromatin is very frequently found in the cytoplasm of protozoa. Givago states, that the sexual development in the microsporidia consists in chromidiogamia in all definitely known cases. Here the nuclei of copulating individuals lose their outlines, the nuclear chromatin is transformed into chromidia and the copulation of the nuclear substance takes place at the time, when it is scattered in the cytoplasm.

Again after the union of the gametes, Popoff finds, that the nuclear substances transfuse into the cytoplasm, accumulate around the nucleus and are transformed into a chromidial net. According to Popoff, Svarchevsky and Elpatievsky this chromidial net gives rise to the nuclei of the gametes and also to the nuclei of the Shisogonic descendents of Arcella. A description of diffuse scattering of chromatin substance in the cytoplasm of the protozoa is recalled by the names of Schaudinn and Hertwig. Some of the figures of Dobell's paper, which represent the development of Adelae ovata exactly correspond to some of my preparations of parthenogenetic eggs in the prophase of the first cleavage.

This analogy between the structure of unicellular organisms, during their multiplication, and that of multicellular organisms in the unicellular stage may offer some further suggestion. The students of protozoa identify the chromidial nets and the chromatin granules scattered in the cytoplasm with true nuclear chromatin. They seem to be justified in doing that, the nucleus· of the protozoa usually losing its individuality; yet the nucleus in the egg cell of the sea urchin, on the contrary, retains its outlines, while the cytoplasm contains the described chromatic accumulations. Therefore, great caution is required in the interpretation of the chromatic accumulations found in the cytoplasm of sea urchin eggs.

4. FIRST CHANGES IN THE CYTOPLASM OF NORMALLY FERTILIZED AND PARTHENOGENETIC EGGS

The first changes in the structure of the egg after normal fertilization and artificial parthenogenesis are similar, both in the formation of the membrane and also in the appearance of deeper changes, which take place in the cytosome. As already mentioned, the characteristic structures of the mature egg are: a small achromatic spherical nucleus and numerous basophilic accumulations in the cytoplasm. These accumulations appear as peculiar spherical bodies. They may remain unchanged for many hours in mature unfertilized eggs. The oxidation rate in the unfertilized egg being low, the morphological structures remain stable.

With the penetration of the spermatozoon into the egg the distribution of the basophilic substance in the cytoplasm undergoes conspicuous changes. The larger accumulations invariably become smaller and the basophilic substance is soon much more uniformly distributed in the cytoplasm. These conditions seem to be of importance, because they occur with equal invariability in both the normally fertilized and the parthenogenetic eggs (fig. 3 and figs. 4, 5, and 6). In both cases the stable equilibrium of the different parts in the cell seems to be disturbed and latent potentialities become apparent.

Loeb finds in fertilization and egg development an acceleration of oxidation processes, and believes the synthesis of the nuclear substance to be effected at the expense of the cytoplasm. He admits, that the nuclear substance becomes catalysor in the process of chromatin synthesis. The conception of synthesis of chromatin at the expense of cytoplasm, first clearly expressed by Loeb, is of great importance in the development of living matter.

The specific stimulus of the latent potentialities of the egg cell is the spermatozoon, and all its constituent parts have been in turn regarded as of exclusive significance in fertilization. The discovery of artificial parthenogenesis by Delage and Loeb have shown that, in reality, no part of the spermatozoon has such exclusive

significance, and that the development of the ovum can take place without participation of the spermatozoon. The fertilization, or rather the calling forth of development, must necessarily consist in activation of chromatin synthesis at the expense of the cytoplasm. These processes may be related to morphologically appreciable scattering of the basophilic accumulations, and, as shall be seen later, to a flowing of the chromatic substance to the achromatic frame-work of the nucleus.

The natural stimulation of the egg to development is a complex process. The presence of the spermatozoon within the ovocyte not only incites the latter to development, but also induces complex chemical changes, which as their consequence have a partial transmission of the paternal hereditary qualities and perhaps also the development of new qualities. And yet, different as the normal and artificial stimuli may appear, they both incite similar, changes in the ovum. These changes concern a very exact morphological process—a diffuse scattering of the basophilic substance in the cytoplasm. Whether this diffusion results only in the flowing of the basophilic substance to the nucleus, or whether this process may have a connection with the synthesis of the chromatic substance is difficult to determine.

A closer study of the preparations gives no indications of a fragmentation of the larger masses, as such. The large basophilic accumulations appear invariably immediately after normal fertilization and in the beginning of parthenogenetic development, surrounded by a more diffuse zone of intensely staining cytoplasm. It seems that the chromatic masses formerly clearly defined now undergo a gradual dissolution, and thereby impregnate the surrounding cytoplasm. The cytoplasm around the chromatic accumulations exhibits a pronounced reticular structure, some of the meshes of the reticulum being arranged radially to the chromatic accumulations. The diffusion of the chromatic accumulations in normal fertilization is accomplished slowly, but effectively and finally leads to quicker development than during parthenogenesis. The chromatic substance, which now more diffusely impregnates the cytoplasm, does not remain in fertilized eggs in loco, but in its new physical state is readily

transported. The impregnation of the cytoplasm seems to proceed only to a certain degree. In the case of excessive impregnation it becomes reconstituted in morphologically visible basophilic particles.

The basophilic chromatic substance, distributed now more or less uniformly in the cytoplasm, shows definite relations to the achromatic nucleus. A radial arrangement of the cytoplasm now becomes evident (fig. 3) around the segmentation nucleus. This radial arrangement seems to be caused by the movement of the basophilic substance from the cytoplasm to the nucleus, and is very conspicuous in the normally fertilized eggs as well as in regularly developed parthenogenetic eggs. Accumulations of diffuse basophilic substance may appear around the nucleus as seen in figure 3. Less regular is the accumulation in figures 4 and 6. Well pronounced circum-nuclear zones of basophilic substance appear however infrequently in normally fertilized eggs. Figures 14, 15 and 19, represent nuclei of normally fertilized eggs with adjacent cytoplasmic zones and show only slight accumulations of basophilic substance around the nuclei.

The process of dissolution may concern all the basophilic accumulations at the same time. Again, other eggs exhibit this process only in the immediate vicinity of the nucleus. As result in the latter case, a zone of cytoplasm around the nucleus becomes freed of all discrete masses of this substance. At the periphery of such eggs many small reconstituted granules may be observed (fig. 3). A characteristic feature of the small granules is their location at the intersection of cytoplasmic reticulum.

The described process of diffusion and subsequent movement of the basophilic substance to the nucleus occurs in a very different manner in parthenogenetic eggs. The chemical stimulation of the egg may incite in the beginning of development chemical changes similar to those observed in normally fertilized eggs, but artificial irritation is incomparably rougher, (at least by Delage's method). If the chemism of the development is in principle similar in normal fertilization and in partheno-

genesis, it proceeds in the latter case much more vigorously and leads to such peculiar formations as the artificial cytasters.

Every investigator, who has studied artificial parthenogenesis, is familiar with the appearance of cytasters. Morgan described them in 1896, '99, and '90; Wilson in 1901. According to Wilson, they may be found in different parts of the cytoplasm. The primary radial arrangement of the cytoplasm around the nucleus in such cases is less strongly marked. These cytasters may function as centers for division, but no full division of cytoplasm occurs around cytasters, which do not contain chromosomes. The cytasters may contain in their centers deeply stained central corpuscles. Wilson identifies the central corpuscles of the cytasters with true centrosomes and concludes, like Morgan, that both of them are formed de novo. It is well known what a severe protest this opinion drew from Boveri. According to Boveri, Morgan in his experiments obtained, besides transitory artificial astrospheres, which does not participate in mitosis, only pathologically multiplied ovocentra and their derivatives. Fischer and Oswald explain the formation of astrospheres during artificial parthenogenesis through a coagulation produced by withdrawal of water.

The relation of the cytasters and the centrosomes will be discussed later. At present merely the origin of the artificial cytasters will be studied—as judged by the conditions found in my preparations.

As mentioned above, the chromatic basophilic bodies become labile, impregnate the surrounding protoplasm and cause the network of cytoplasm to be more intensely stained around them. The single meshes of the cytoplasm become thereby thicker and appear sometimes in the form of a few radiations, directed toward the basophilic centers. This may be observed occasionally in normally fertilized eggs, in which the basophilic substance is gradually transported to the remote parts of the cell. Should the dissolution of all the chromatic masses in parthenogenetic eggs occur rapidly and simultaneously, the rapid flowing off of this substance will at the same time transform numerous meshes into radial strands (fig. 5). As in normal fertilization, this sub-

stance seems to be attracted by the apparently achromatic nucleus, but in its movement encounters a great obstacle in similar strong currents directed from other chromatic accumulations. As result of these processes, many small cytasters appear. If they are not numerous, they gradually disappear, because of the flowing of the substance to the nuclear region.

No doubt many cytasters contain a basophilic and intensely staining corpuscle in their center. These corpuscles sometimes appear as very distinct granules; again they are sometimes diffusely stained, and do not show precise limits. Some of the cytasters do not contain any such central bodies, yet there is no other distinction between the different kinds of cytasters.

The appearance of artificial cytasters may be accounted for by the existence of physico-chemical processes similar to those found in normal fertilization. and seems to depend upon a more vigorous action of the same forces. The study of artificial cytasters invariably indicates that a radial arrangement of the cytoplasmic meshwork appears, when currents are present, which transport certain substances from one part of the cytoplasm to the other. A radial arrangement of the cytoplasm appears when the basophilic substance is transported toward the nuclear region. This radiation has the nucleus as its center and is formed by a centripetal current. More or less well pronounced radiations may be formed by centrifugal currents and result from the dissolution of the basophilic accumulations. All these radiations are temporary re-arrangements of a common structural basis of the cytoplasm, become less pronouned and finally gradually disappear, when the currents exist no more.

A very interesting modification in the distribution of the basophilic chromatic substance is brought about by a special anomaly in the nucleus, occasionally observed in the very early stages of parthenogenetic development. Sometimes the action of the chemical substances applied manifests itself so intensely, that the achromatic part of the nucleus breaks into two, three, four and more achromatic regions, which again assume a spherical form. Sometimes these nuclear parts remain close together, (fig. 22), in other cases they appear separated (figs. 5 and 20).

Loeb considers the fragmentation of the nucleus as result of
the influence of too strong solutions and calls it amitotic disin-
tegration of the nucleus. If such nucleus fragmentation takes
place at a time, when chromatic basophilic substance is still pres-
ent in the cytoplasm, every nuclear part continues to exert at-
traction of the chromatic substance, and the latter accumulates
around every little part of the achromatic nuclear substance
(figs. 20, 22). In such cases many isolated radiations may ap-
pear around the nucleus particles. They are formed by centri-
petal currents, and are similar to the single radiation in the fer-
tilized egg. In some of the eggs there could not be traced in the
whole series of sections any noticeable part of the nucleus, and
yet their cytoplasm contained many cytasters. Should we con-
clude in such cases that the substance of the achromatic nucleus
has been scattered by the violent action of the chemical agent
through the cytoplasm in the form of small particles, and that the
cytasters developed around them? Much less evident are at
this time the changes connected with the structures, which
correspond to plastosomes.

5. FIRST CHANGES IN THE EGG NUCLEUS AFTER FERTILIZATION AND IN PARTHENOGENESIS

The small achromatic nucleus, as shown above, seems to exert
strong attraction of the chromatic basophilic substance, which
is followed by its displacement. The next changes in the mutual
relationship of the achromtic nucleus and the basophilic sub-
stance of the cytoplasm are more conspicuous and therefore
easier to study in parthenogenetic eggs.

The normally fertilized eggs present at this time the complex
picture of the union of two pronuclei, and, at least in some cases,
the egg pronuclei at once gets a considerable amount of chro-
matic substance from the spermatozoon (figs. 14, 15, 17). On
the contrary, parthenogenetic eggs show, step by step, the
gradual development of chromatin and chromosomes in the
nucleus. Moreover, parthenogenetic eggs often show certain
anomalies in the development of chromatin and chromosomes,
which may greatly facilitate its interpretation. Therefore a

study of chromatin differentiation will be made in parthenogenetic eggs first.

Figure 7 shows a nucleus of a mature, unfertilized ovum under high magnification. In all three sections, which contained parts of the egg no chromatin granules whatever could be traced within the nucleus. The highest magnification did not disclose in it any definite structure except that it seemed to be finely reticular or finely granular. Some of the nuclei of other unfertilized eggs contain one or two intensely stained granules closely adjacent to the membrane and even flattened upon it (fig. 2). Those granules within the nucleus show the same staining reaction and the same morphological appearance as the chromatic accumulations in the cytoplasm. A well marked membrane is characteristic of the nucleus of an unfertilized egg. Obst and Conklin have described continuity in a resting nucleus between the cytoplasmic reticulum and the network of the nucleus. Unfertilized eggs do not show connections between the nucleus and the cytoplasm, but connections between the cytoplasm and the nuclear membrane may be easily traced in developing eggs.

Figures 8, 9, 10, 11, 12 and 13 represent sections of parthenogenetic eggs. An interesting feature, common to all these eggs, is the formation of intensely staining particles immediately within its nuclear membrane. This chromatic basophilic substance, which appears within the nucleus and which without doubt contributes to the formation of chromosomes, may certainly be called chromatin.

A comparison of the nucleus of an unfertilized egg on figure 7 with the nucleus of an egg in the beginning of parthenogenetic development on figure 8 indicates merely slight changes around the nucleus on the figure 8. The nuclei on both figures are nearly equal in size; they both maintain a distinct membrane. The only difference between them consists in accumulations of basophilic granules within and without the membrane of the nuclei. The presence of the nuclear membrane allows to easily recognize which of the chromatic granules lie within the nucleus and which of them are in the cytoplasm. Many granules lie immediately in contact with the nuclear membrane; but most interesting are

those granules which lie partly outside and partly inside of the nucleus, both parts being flattened against each other and separated only by the membrane. The smallest chromatin particles of the nucleus appear as thickenings of the membrane (fig. 8).

The next change in the nucleus is its growth, while its substance becomes still more achromatic and nearly unstainable in my preparations; only under the highest magnification a slight granularity is perceptible. With further development the nuclear membrane loses its distinct outlines, and there appear innumerable connections between the cytoplasmic net and the membrane in the form of thin threads. In eggs, of which the nucleus is surrounded by a larger zone of denser basophilic cytoplasm, these connections appear very clearly (figs. 9 and 11). The substance of the nucleus, on the contrary, remains well separated and different from the cytoplasm; it may resorb substances from the cytoplasm; but once within the nucleus, the resorbed substances do not leave it.

The amount of chromatin inside the nucleus gradually increases, and the granules keep their characteristic position on the inner side of the nuclear membrane for some time. Occasionally they cover the whole inner side of the membrane with innumerable small protuberances (figs. 9 and 13); but their distribution, their form and size, even the intensity of their basophilic reaction may considerably vary. The figures 8, 9, 10, 11, 12 and 13 will demonstrate this. Sometimes single chromatic particles grow very rapidly, attain a large size, (figs. 10, 11 and 13), and appear as characteristic nucleoli, which, in the normal fertilization, seem to have a more important and definite rôle.

As mentioned above, the flowing of the chromatic substance within the parthenogenetic eggs proceeds with great intensity. Around the nucleus (fig. 4), or around parts of the nucleus, (figs. 20 and 22), considerable amount of chromatic substance may accumulate. Since the impregnation of cytoplasm by chromatic substance seems to present certain limits, and its further transformation and movement is not infrequently hindered, it happens sometimes, that the chromatic substance accumulates around the achromatic parts of the nucleus and here becomes recon-

stituted in small, intensely staining granules. Such accumula-
tions of achromatic granules are numerous in figures 4, 11, 13,
20 and 22. The nuclei of these eggs contain numerous chroma-
tin granules. Connections between the granules lying outside of
the nucleus and the chromatin of the nucleus itself can be seen
in figure 4. These connections are effected by numerous threads
of basophilic substance, partly forming a loose net within the
nucleus.

The close relations between the chromatin of the nucleus and
the basophilic substance in the cytoplasm can be easily traced.
The nucleus on figure 10 offers also an example of these close
connections. Its membrane has almost completely disappeared
and a part of the cytoplasm, surrounding the nucleus, appears
strongly condensed and basophilic. On the side of the cyto-
plasm this condensation is directly connected with the network
of the cytoplasm, on the other side it joins directly the large
nucleolus. Figure 11 represents the same conditions in no less
characteristic way. There appear in two different places around
the nucleus linear condensations of cytoplasm intensely impreg-
nated with basophilic substance. Numerous cytoplasmic threads
unite these condensations with the rest of the cytoplasm as well
as with the chromatin particles lying within the nucleus. How
different these condensations may appear is shown by the figures
10, 11 and 12.

Did the study of the differentiation of chromatin in the nucleus
of parthenogenetic eggs contribute some information about its
origin? At the beginning of parthenogenetic development the
nucleus is deprived of differentiated visible chromatin. The
basophilic chromatic substance, accumulated in the cytoplasm,
seems to be gradually transported to the region of the nucleus.
At the same time the nuclear membrane begins to disappear and
the limits between the nucleus and the cytoplasm are finally
effected merely by the difference of the two substances. The
first chromatic particles in the nucleus appear in closest relation
to the cytoplasm, and at first they appear as small, deeply stained
spots at the membrane itself. These spots grow gradually and
become convex toward the center of the nucleus. The nucleus

is surrounded at this time by a dense net of cytoplasm, often intensely impregnated with chromatic substance, and after disappearance of the membrane is in immediate contact with it. All these facts suggest the idea, that the chromatin within the nucleus is differentiated at least partly at the expense of the basophilic substance of the cytoplasm.[1]

Far from insisting upon identity of the chromatin within the nucleus and the chromatic accumulation found in the cytoplasm of immature eggs, I cannot but take into consideration their intimate relations. Possibilities of interaction between the basophilic chromatic substance and the cytoplasm are great while the latter is transported to the nucleus. Incorporated within the nucleus, this substance again may undergo chemical changes. Is it bound chemically to the achromatic substance of the nucleus? A definite answer to these questions is hardly possible at present. The attraction of cytoplasmic chromatic substance by the karyoplasm, its incorporation within the nucleus, its further participation in the development of chromosomes—all these facts seem to inform about complex chemical interchanges between the constituent parts of the cell.

Up to the present the chromatin and especially the reproductive chromatin has been considered as of exclusively nuclear origin. The question of chromatin synthesis has been somewhat neglected. Where, and at the expense of what does chromatin synthesis take place? The loss of staining capacity by the chromatin in a resting nucleus has been attributed to its chemical change. The individuality or even the immutability of chromosomes has been chiefly suggested by Boveri's observations. Boveri described in the resting nuclei of Ascaris eggs protuberances, which correspond to the form of chromosomes, and found that the new formed chromosomes at the next prophase appear in these proturberances.

The small achromatic female pronucleus in the sea urchin eggs does not offer any evidence for the persistence of chromo-

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[1] I am indebted to Prof. David H. Tennent for the opportunity of studying some of his preparations of different parthenogenetic sea urchin eggs. They show a similar development of the first chromatin particles in the nucleus in close connection with its membrane.

somes. It appears homogeneous and achromatic and if it still contains the substance of chromosomes, this substance is chemically changed and is no more chromatin, which once formed the chromosomes. Moreover, if the number of chromosomes be doubled at every mitosis without loss of size the amount of the chromatin must be doubled. In any event we have to conclude that the synthesis of chromatin, even if it does take place within the nucleus, must be effected at the expense of a material derived from the cytoplasm. And the facts of direct observation speak strongly for resorption of definite basophilic chromatic substance by the nucleus from the cytoplasm.

This absorption can lead at different stages to various morphological structures. Groups of small basophilic granules may accumulate around the nucleus, and become connected with the granules within the nucleus by threads; they seem to discharge their substance into the nucleus; again cytoplasmic condensations appear around the nucleus and chromatin particles arise in the nucleus in the form of innumerable small granules, in the immediate vicinity of the cytoplasm; or finally the resorption by the nucleus does not proceed over the whole periphery of the nucleus, but is chiefly localized at one or two points of it and a considerable body of chromatin is formed. (see figures 8, 9, 10, 11, 12 and 13). The nucleus often remains spherical, the radiation around the nucleus is single and no centrosome is seen in eggs while the resorption is proceeding over the whole periphery of the nucleus.

More complicated is the differentiation of the chromatin in the nucleus during normal fertilization and more difficult is its study. The process of chromatin development in a normal egg is more complex because of the copulation of the pronuclei, at which time the segmentation nucleus usually receives a considerable amount of differentiated chromatin from the spermatozoon. The copulation of the pronuclei can procceed in different ways. Sometimes the pronuclei join together very quickly and the chromatin of the spermatozoon is added to the female pronucleus entirely and unchanged (fig. 14); again the spermatozoon, before the union with the female pronucleus, may change into an achro-

matic male pronucleus, and both pronuclei unite in very similar
condition—an achromatic segmentation nucleus resulting from
this union (fig. 16). Finally the male pronucleus may have dif-
ferentiated its chromosomes before union proceeds and during
copulation it adds to the female pronucleus already differen-
tiated chromosomes (fig. 17).

The differentiation of the chromosomes is easier to follow in
cases, where the nuclei join together in an achromatic stage. It
proceeds then similarly to what is seen during parthenogenesis (fig.
16). The membrane of the segmentation nucleus is thin if it
exists at all. The first appearance of the chromatic particles
occurs invariably in the immediate vicinity of the membrane;
they appear in section as lines, grow very rapidly to spherical
bodies, flattened against the membrane, with marked convexity
toward the center of the nucleus. Figure 16 shows the forma-
tion of larger bodies which remind the nucleoli. On the cyto-
plasmic side these nucleoli are surrounded by a denser cytoplasmic
net.

The conditions within and without the nucleus must be dif-
ferent at this time, for the chromatic substance, once it has
entered the nucleus, does not again leave it. The nucleolus con-
tinues to grow by the side next the cytosome. At the same
time its substance becomes loose on the opposite side. This
loosening of the nucleolus is quite similar to that of the sper-
matozoon, when it joins the female pronucleus in a dense state.
A comparison of figures 14 and 16 will show, that at the peri-
phery of the nucleolus as well as at the periphery of the spermato-
zoon, numerous threads appear, which grow toward the center
of the nucleus.

The figures 14, 15 and 17 illustrate the resorption of chromatic
substance by the segmentation nucleus in cases, when the male
pronuclei add to the female pronuclei considerable quantities of
chromatin, either in an unchanged form (fig. 14), or in the form
of already differentiated chromosomes (fig. 17). The resorption
of the cytoplasmic basophilic substance proceeds partly in the
form of numerous small spots on the membrane, partly in the
form of one or of two well differentiated nucleoli.

A striking difference is presented by the form of the nucleus after copulation of the nuclei and in artificial parthenogenesis. In normal fertilization it is always oval: in the parthenogenetic egg, nearly always spherical. I am not able to explain this difference or even to trace its causal relation to any other fact. The nucleus of a normally fertilized egg is oval at the time, when a single uniform radiation surrounds it (fig. 14); the nucleus is oval, when the radiation is bipolar (fig. 16). Since the poles of the nuclei are often occupied by nucleoli, it seemed natural to think of a possible relation between the form of the nucleus and the position of nucleoli. If, however, the coefficient of surface to mass may offer in the zones of greater convexity of the nuclei more favorable conditions for resorption, the form of the nucleus would be the primary factor. Large masses of absorbed substance appear indeed in the zones of greater convexity as the sequence of the oval form of the nucleus, yet these accumulations of chromatin may also be formed in other parts of the nucleus, as figures 15 and 18 show.

The oval form of the nuclei appears soon after copulation of the pronuclei and the plane of their union has no relation to either diameter of the oval segmentation nucleus. The nucleus of figure 14 is oval; the plane of union being in this case parallel to its longer diameter but the longer diameter of the nucleus is often perpendicular to the fusion plane. It might be asked whether the change in the form is possibly due to a specific influence of the centrosomes. Yet immediately after copulation there could not be found at the poles of innumerable eggs studied any structures resembling centrosomes. Neither is it possible to trace a causal connection between radiations and nucleus form, and if such exists the form of the nucleus must be considered again as the primary factor. The single radiation, which develops around the segmentation nucleus (fig. 14), is invariably followed by a rearrangement of the cytoplasm into a bipolar radiation around the two poles of the oval nucleus (fig. 16).

6. DIFFERENTIATION OF CHROMOSOMES, OF THE SPINDLE AND OF THE CENTROSOME

The study of parthenogenetic development in the sea urchin eggs has been of great help in the study of chromatin differentiation in the nucleus. The further development of parthenogenetic eggs, the differentiation of chromosomes, of the spindle and of the centrosome, becomes often very irregular. The anomalies observed in transportation and resorption of the chromatic cytoplasmic substance influence the further development of the egg and result in complex changes, which concern at the same time different cell structures. Parthenogenetic eggs exhibit during the first cleavage numerous and various anomalies in the development of the mitotic figure, while in normal development the corresponding structures show a perfect regularity. The further observations on the cell organs will therefore be based principally on the study of normally fertilized eggs.

The development of chromosomes is closely connected with that of the spindle and of the centrosome. According to differences observed in the resorption of the basophilic substance and in the copulation of the pronuclei, the development of the chromosomes often exhibits a great diversity. The individual variations in the development of the chromosomes are so numerous, that their description would require a separate study. A few examples are sufficient for my purpose:

If the spermatozoon joins the female pronucleus in a dense condition, which seems to be typical, its chromatin becomes gradually looser within the segmentation nucleus. A meshwork of chromatin threads results, which soon unites with chromatin particles of the female pronucleus. Figure 15 illustrates similar connections between the paternal and maternal chromatin. A common network is gradually formed, from which the chromosomes are differentiated.

Should the spermatozoon have changed its dense structure and have become achromatic before the fusion of the pronuclei took place, the segmentation nucleus in such cases appears almost free of chromatin (fig. 16). The development of chromatin within the achromatic segmentation nucleus will take place after

copulation. Chromatin particles appear in connection with the nuclear membrane as numerous small, or a few large, accumulations. The small accumulations may become free from the nuclear membrane and may be found within the achromatic substance of the nucleus as well defined bodies (fig. 19). Offshoots in form of thin threads are seen to grow from such chromatin bodies (fig. 19); they form a chromatin net, from which the chromosomes are differentiated (fig. 28).

Less frequently the fusion of the pronuclei takes place at a time when the male pronucleus already possesses differentiated chromosomes. No common net of chromatin is formed in this case and the sperm and egg chromosomes remain entirely separate during the whole time of their differentiation.

As mentioned above, a bipolar radiation finally appears around the two poles of the nucleus. The single radiation around the nucleus seemed to be connected with the flowing of the basophilic substance toward the nucleus. The bipolar radiations may sometimes appear at an early stage, when the segmentation nucleus does not show differentiated chromatin. In other more numerous cases chromosomes are in full development or even already formed before the bipolar radiations appear. The bipolar radiations seem to be connected with the development of the spindle, since the threads of the spindle begin to grow usually from the poles of each of the radiations. The larger portion of the radiation or astrosphere is formed by the elongated meshes of the cytoplasm. The sector of the radiation known as the spindle seems to be formed by the substance of the plastosomes, (M. Lewis, Robertson), or archoplasm (McClung), and consists of uniform thin threads (fig. 26). It is transported to the poles of the nucleus by currents, which again transform the cytoplasm into radiations appearing this time as bipolar. The cytoplasm becomes denser at the poles of the nucleus where the radii of the radiation converge. In these regions appear the structures known as centrosomes and centrioles.

The poles of the nucleus during the growth of the spindle show frequently a marked depression. The whole nucleus sometimes may be compressed so energetically that it becomes oval in the

direction opposite to the former. Is the growth of the spindle influenced by the achromatic part of the nucleus or by the chromosomes, which at this stage are in full development? The attachment of the spindle fibers to the chromosomes seems to speak (McClendon, McClung) for the existence of a pronounced mutual attraction between these structures. The appearance of the spindle within the nucleus influences the distribution of the chromosomes. These latter are pushed by the growing spindle to the equator of the nucleus, where they become situated at one level, forming the equatorial plate of the mitotic figure. At this stage the differentiation of the chromatin into the chromosomes and of the plastosomes into the spindle attains its culminating point.

Both in cleavage and in division of somatic cells a supreme importance has been attributed to the centrosome. Boveri considers the centrosome as an organ, from which the impulse to cell division arises. Though many details of cell division are now known, the principle of this elementary process is not yet recognized. With the discovery of the centrosome a ray of light seemed to penetrate the field of cell division studies. But unfortunately this ray was soon taken for the source of the light, for the prime and final cause of cell division. To Boveri the centrosome appeared as a self propagating cell organ which supplied the dividing cell with mechanical regularity. This view found support in the papers of many biologists. And though Boveri himself does not regard the centrosomes of equal importance with the chromosomes, some of his followers went, in their deduction, much further than he.

For a while the importance of the centrosome was strongly emphasized, but in the face of cumulative adverse evidence it can no more be regarded as a permanent cell organ (Lillie, Child, Wilson, Morgan, Hertwig, Klinkowström). My own results strengthen this conclusion.

The centrosomes first appear in my preparations at the poles of the nucleus. Their differentiation proceeds locally, without any relation to the centrosomes of the spermatozoon or to the primary single radiation around the segmentation nucleus. The

bipolar radiations are here, as elsewhere, signs of movement and currents in the cytoplasm, which are stronger, at this time, at the poles of the nucleus, where the relative surface of the absorbing membrane is greater. As above stated, the segmentation nucleus becomes oval. At the time of the development of the bipolar radiations the basophilic chromatic substance is usually in greater part absorbed by the nucleus. Therefore, the bipolar radiations are not due to the movements of the cytoplasmic chromatic substance. Their appearance seems to be connected with the shifting of a substance corresponding to the plastosomes of Meves and Duesberg, or to the archoplasm of McClung and other authors. Accumulations of archoplasm around the poles of the nuclei contribute to the formation of the structures known as centrosomes and centrioles. These cell organs, if they may be so called have a definite time limitation for the beginning of their existence. No relation between the centrosomes of the segmentation nuclei of the sea urchin eggs and the centrosomes of the spermatozoon can be traced. Lillie's interesting experiments seem to strengthen this position. Lillie recently centrifuged fertilized eggs, whereby merely small parts of the spermatozoon heads remained in the egg, a considerable part of the head, the middle piece, and the tail having been withdrawn. These eggs continued to develop and centrosomes differentiated. The centrosomes of the first segmentation nucleus do not appear in well fixed preparations as independent bodies. They appear, on the contrary, as dense cytoplasmic nets, in which granules sometimes accumulate (fig. 26). The centrosome is usually small, while the spindle develops.

When the spindle is fully developed, and the chromosomes, form the equatorial plate the cytoplasm itself does not contain any such differentiated structures as plastosomes or chromatic granules. The stage of the equatorial plate is the expression of physico-chemical reactions fulfilled between the achromatic substance of the nucleus and the absorbed chromatic cytoplasmic substance and further between the differentiated chromosomes and the archoplasm growing into the nucleus. How intimately connected at this time are the spindle and the chromosomes, is

shown by the experiments of McClendon. The chromosomes seem to be firmly attached to the spindle since the electric current compels the whole spindle to move together with the chromosomes. This is also shown in Hill's work, and that of Conklin and others on centrifuged eggs. The eggs of the sea urchin, during the metaphase, do not present a material particularly favorable for the study of the splitting movements of the chromosomes. These are 36 in number, small and compactly drawn together. The data about the further development are not precise enough to allow definite conclusions.

Similar, but less regular, is the development of chromosomes of the spindle, and centrosomes in artificial parthenogenesis. Parthenogenetic eggs, in which the nucleus has not been damaged by application of too strong solutions, exhibit a regular development. Even artificial cytasters may disappear, the chromatic substance finding finally an outflow to the nucleus. Absorption of chromatic substance proceeds in the usual way and chromosomes are differentiated within the nucleus.

The study of anomalous development of the egg incited by the breaking up of the achromatic nucleus into more or less numerous parts is very instructive. The egg presents in this case several regions of attraction of the chromatic substance. The differentiation of chromosomes cannot proceed regularly, as shown on figures 20 and 22. This injurious influence of fragmentation of the nucleus on the differentiation of chromosomes indicates how important is the rôle of the achromatic substance of the nucleus in the differentiation of chromosomes. Yet it seems as if the achromatic nucleus be not capable of forming the chromosomes by itself. Eggs which do not present the above described dissolution of the chromatic accumulations do not develop. The achromatic nucleus may not present any injury, no chromatin particles are formed within the nucleus. Such eggs can remain in the culture from six to eight hours without showing any morphological changes.

The differentiation of the chromosomes in the nucleus of parthenogenetic eggs proceeds in a manner similar to that of normal fertilization. The morphological pictures are just as

various as they are in normally developing eggs. If during resorption of the cytoplasmic basophilic substance numerous small chromatin particles are formed at the inner periphery of the nuclear membrane, soon some of these chromatin particles become larger, others free themselves from the membrane, connect together by outgrowing filaments, and finally form a chromatin net. If the resorption is more intense in some parts of the nuclear surface, larger chromatin bodies are formed, which contribute also to the development of the chromatin net (fig. 21). It has already been mentioned that complex chemical interchanges must take place between the achromatic part of the nucleus and the absorbed basophilic substance, the final results of which are represented by the differentiation of chromosomes.

Some interesting changes are undergone by the archoplasm or plastosomes in artificial parthenogenesis. There are frequent cases in which the plastosomes show regressive changes, sometimes they are discovered in the cytoplasm, but not attracted by the nucleus. In other cases they flow very uniformly towards the whole periphery of the spherical egg and form here a single radiation. In such cases no bipolar radiations arise, nor a spindle. This is the case of a typical monaster without division of the nucleus. The chromosomes are differentiated, but no movement of the chromosomes follows. Another cycle of differentiation of chromosomes begins (fig. 23), without division of the nucleus. Evidently the division of the nucleus requires a participation of plastosomes or archoplasm in the development of the spindle.

In some eggs the archoplasm accumulates irregularly around the nucleus and from the centers of its accumulations grows within the nucleus in the usual form of threads which are fastened to the chromosomes. In such cases numerous centers arise at once, to which the chromosomes are transported. The partial movement of the chromosomes forms sometimes very typical half spindles.

Finally another interesting anomaly in the formation of the spindle may be observed. The spindle is formed without any precise radiation in the cytoplasm (fig. 25). In this case the

chromosomes are usually very rudimentary and the spindle itself is small.

It is only natural that, anomalies in the development of the spindle be accompanied by anomalies in the centrosphere, since the centrospheres appear as parts of cytoplasm, in which the rays of the bipolar radiations converge and from which the threads of the spindle are growing into the nucleus. With the regular flowing of the archoplasm to the whole circumference of a spherical nucleus, no centrosome will differentiate, a condition which occurs in the case of a monaster. With several irregular accumulations of the plastosomes around the nucleus, several rudimentary centrosomes arise at once. With the development of a small spindle without cytoplasmic radiation no centrosomes are seen on their poles. These cases of development of spindles without centrosomes recall the mitotic figures of the plant cells.

The further division stages consist, according to the usual description, in simultaneous enlargement of the centrosomes, shifting of the chromosomes, and in the consecutive changes of the chromosomes in vesicles and in their mutual fusion into daughter nuclei (figs. 29, 30 and 31). The daughter nuclei again appear achromatic (fig. 31). This must depend upon chemical changes in the substance of chromosomes, yet it is not possible to determine, whether these changes depend merely upon absorption by the chromosomes of certain substances from the cytoplasm or whether a reciprocal exchange of certain substances between them and the cytosome is involved. The ultimate fate of the spindle substance is more or less definite. The spindle fibers, like the chromosomes, experience equal division and soon afterwards disperse themselves uniformly in the cytoplasm of the two daughter cells.

At the time of the reconstruction of the daughter cells the cytoplasm divides. Around the achromatic daughter nuclei single radiations arise, which again are visible manifestations of cytoplasmic currents and which contribute to a re-grouping of different substances in the cell. These currents are directed toward the nucleus. The attraction exerted by the two daughter nuclei creates a zone in the cytoplasm of the egg, in which the

attraction by the two nuclei works in opposite directions, and a division consequently takes place in this zone.

The subsequent development of each of the daughter cells is similar to the development of the mother cell, as are similar the starting points of their development. Each blastomere (fig. 31) has an achromatic nucleus, surrounded by more intensely staining cytoplasm; each blastomere contains numerous small basophilic granules in the cytoplasm. Several small chromatin particles appear at the inner side of the membrane; they grow and form a chromatin net, at the expense of which the chromosomes are differentiated. A concentration of the archoplasm (plastosomes) at the poles of the nuclei is effected through bipolar radiations and the ingrowth of this substance into the nuclear cavity contributes to the formation of the spindle. A study of the first segmentation in the sea urchin egg does not contribute any evidence for the assumption of a primary rôle of the centrosome, nor for its self-propagation. The appearance of the centrosome reveals itself as an important stage in mitotic process, but like the appearance and disappearance of every other cell organ, is closely and necessarily connected with certain physicochemical changes in the egg substance.

7. SUMMARY

1. Accumulations of basophilic chromatic substance are found in the cytoplasm of mature sea-urchin eggs (Strongilocentrotus lividus).

2. After fertilization and at the beginning of artificial parthenogenesis these basophilic accumulations undergo a dissolution. This process is effected in parthenogenetic eggs more vigorously and incites the appearance of cytasters.

3. The small achromatic nucleus of the mature egg both after fertilization and at the beginning of parthenogenesis seems to exert a strong attraction on the basophilic accumulations found in the cytoplasm. This substance is soon displaced and localized around thenucleus. At the same time a radiation around the nucleus is perceptible, which apparently is the expression of the flowing of the basophilic substance toward the nucleus.

If application of too strong solutions is followed by breaking up of the nucleus in a few parts, each of these parts exerts an attraction on the basophilic substance of the cytoplasm and the latter accumulates around them.

4. The development of chromatin within the nucleus in normally fertilized eggs as well as in parthenogenesis proceeds in the form of small spots or bodies, intimately connected with the membrane. The close relation between the true chromatin bodies within the nucleus and the basophilic chromatic substance in the cytoplasm is easily traced. The chromatin of the nucleus may develop either in the form of innumerable small granules, appearing invariably at the inner surface of the nuclear membrane or in the form of larger bodies, which are similar to nucleoli. Chromatin develops in a similar way in parts of the nucleus after application of too strong solutions.

5. The union of male and female pronuclei can proceed differently. The male pronucleus may join the female pronucleus in an achromatic stage; in other cases it adds to the female pronucleus during copulation considerable amounts of chromatin either in unchanged form, or in the form of already differentiated chromosomes.

6. The chromosomes are differentiated from the chromatin network of the nucleus. The chromatin bodies, which first appear at the inner surface of the nuclear membrane, contribute to the formation of the chromatin network; they give offshoots, which soon join together.

7. The differentiation of the spindle seems to be connected with the bipolar radiations and with the migration of plastosomes or archoplasm. The threads of the spindle are attracted by the chromosomes and attach themselves firmly to them. They grow into the substance of the nucleus and push the chromosomes to the equator. Centrosomes at the first cleavage mitosis appear as a dense network in the center of the bipolar radiations or astrospheres, in which network accumulations of basophilic granules may be seen.

8. The chromosomes undergo vesicular transformation during telophase, become achromatic and join together into an achro-

matic daughter nucleus.   The achromatic daughter nuclei exert
an attraction upon the basophilic substance, which again is found
in the cytoplasm.   Radiations surround the nuclei, and a division
of cytoplasm takes place in the region, where the radii of the
astrospheres meet.   In this region the attraction, exerted by the
nuclei, is displayed in two opposite directions.

9. A few statements of more general value may be allowed.   It
seems on the basis of the present study, that if a given structure
appears regularly at definite stages of cell mitosis, it is due to the
fact, that the physico-chemical process of life is composed of
cycles, and that the final stages of cycles become starting points
for other similar cycles.   Every step of a cycle is exhibited in our
preparations by the regular appearance of morphological struc-
tures (cell organs), which as such are all temporary.   They re-
appear regularly as result of development of analogous physico-
chemical processes, which underlie the life history of the cell.
Their changes are easily appreciable in those stages of develop-
ment of living substance, which are separated by lengths of time,
and appear as different specimens of the organic world.   The
differences exhibited by the most essential cell organs are in this
case easily demonstrated by our preparations.   The study of
the gradual differentiation of cell organs during ontogenesis
offers great difficulty and only further investigations may throw
light upon this problem.

It gives me great pleasure to express my thanks to Prof. C.
E. McClung for valuable advice and for reading the test.

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All figures were made with the camera, at stage level, with Zeiss apochromatic 2 mm. oil immersion objective; figures 1 to 6, 24 and 27 with no. 4 compensating ocular, all the others with no. 18. All sections from eggs, preserved in Zenker-formol, except figure 27 drawn from a section of an egg, preserved in Zenker. For a detailed description of the figures see the text.

PLATE 1

EXPLANATION OF FIGURES

1 Immature sea-urchin egg with a large germinal vesicle, containing a deeply stained nucleus, chromatic granules and filaments. The cytoplasm shows the presence of large, dense ribbons, similar to the large plastosomes of the spermatogonia of insects.

2 Mature sea-urchin egg with a small achromatic female pronucleus; the cytoplasm contains large chromatic bodies.

3 Egg 40 minutes after fecundation. Single cytoplasmic radiation around the oval segmentation nucleus; small chromatic granules in the cytoplasm; development of the chromatin in the nucleus in the immediate vicinity of the cytoplasm.

4 Parthenogenetic egg after remaining 40 minutes in sea-water. Single cytoplasmic radiation around the spherical nucleus and accumulation of chromatic granules; development of the chromatin in the nucleus and its connection with the outside laying chromatic material.

5 Parthenogenetic egg after remaining 40 minutes in sea-water. Many astrospheres and fragmented nucleus.

6 Parthenogenetic egg after remaining 1 hour in sea-water; single radiation around the oval nucleus. Chromosomes (drawn from two sections) defectively developed in the nucleus; many small chromatic granules, lying in the cytoplasm.
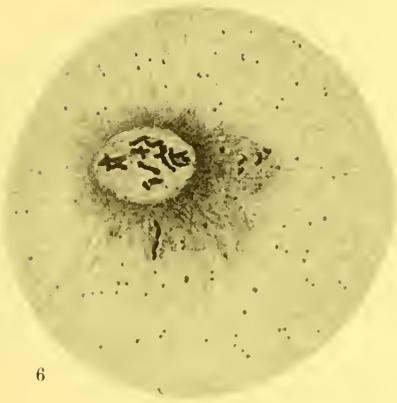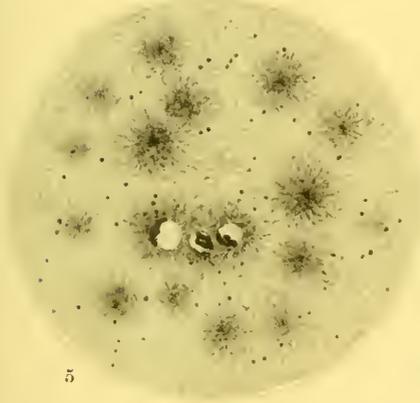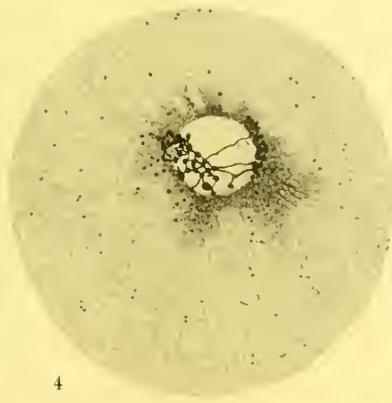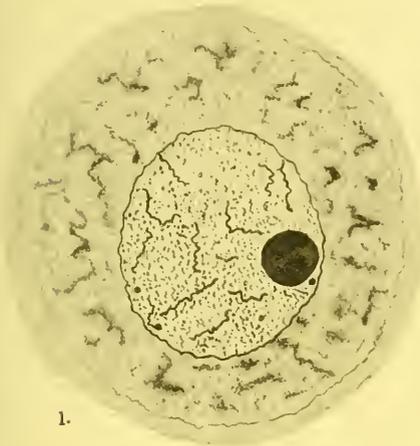
7   Nucleus of an immature egg, with the adjacent cytoplasm.   Does not contain any chromatic substance; large chromatic bodies lying in the cytoplasm.

8   Nucleus of a parthenogenetic egg after remaining 10 minutes in sea-water. Small chromatic granules outside and inside of the nuclei.

9 and 12   Nuclei of parthenogenetic eggs after remaining 15 minutes in sea-water.   Many small chromatic granules adjacent to the nuclear membrane and similar chromatic granules in the cytoplasm.   Figure 12 shows a condensation of cytoplasm, impregnated by a basophilic substance.

10   Nucleus of a parthenogenetic egg after remaining 20 minutes in sea-water. A large chromatic body within the nucleus, directly connected with the cytoplasm, whose threads and meshes are impregnated by a basophilic substance.

11 and 13   Nuclei of parthenogenetic eggs after remaining 35 minutes in sea-water.

11   Chromatic impregnation of the cytoplasm around the nucleus; development of the chromatin net within the nucleus.

13   Nucleoli; numerous small chromatin granules within the nucleus at its periphery; many small chromatic granules in the cytoplasm around the nucleus, partly connected with the granules lying in the nucleus.

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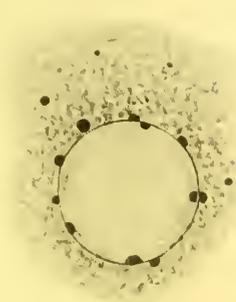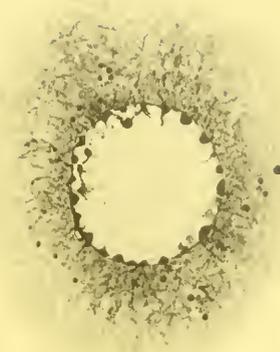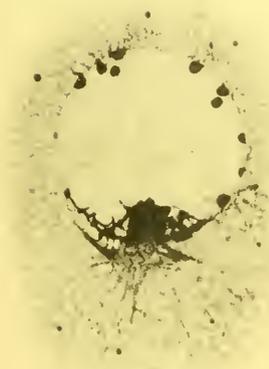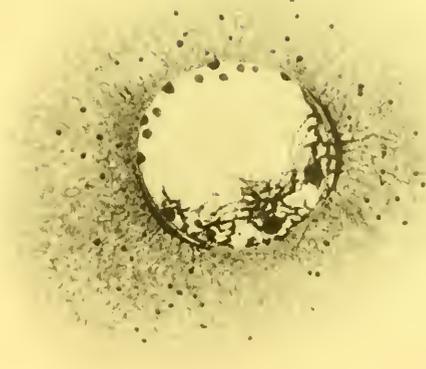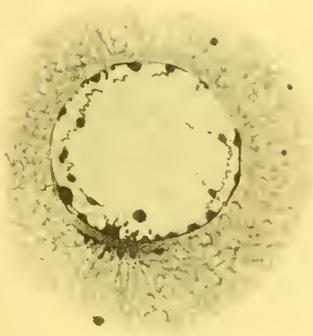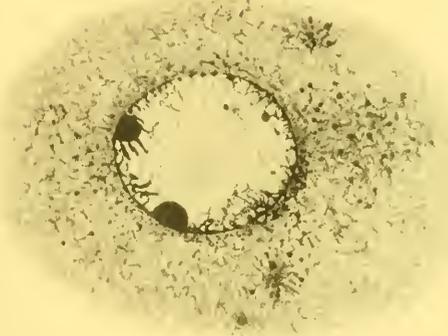
14 Nucleus of an egg 30 minutes after fertilization; spermatozoon loosening its substance within the segmentation nucleus; uniform cytoplasmic radiation around the segmentation nucleus; many small chromatic granules in the cytoplasm.

15 Nuclei of eggs 40 minutes after fertilization. Chromatic substance of the spermatozoon loosened and connected with the nucleolus of the female pronucleus.

16 Achromatic oval segmentation nucleus with two nucleoli at its poles and small chromatin granules at its periphery; radiations around each pole of the nucleus; many small chromatic granules in the cytoplasm.

17 Nucleus of an egg 40 minutes after fertilization. Copulation of the two pronuclei, the male pronucleus containing fully developed chromosomes.

18 Nucleus of an egg 40 minutes after fertilization. Accumulation of the chromatic granules around the segmentation nucleus, rare in fertilized eggs.

19 Nucleus of an egg 40 minutes after fertilization. Outgrowing of filaments from the chromatin granules and development of a chromatin net (next stage in figure 28).

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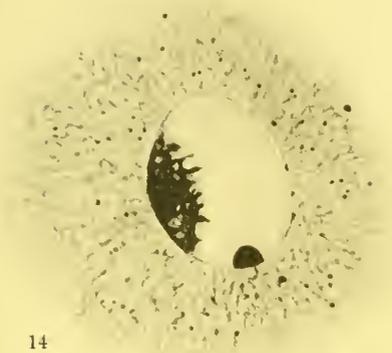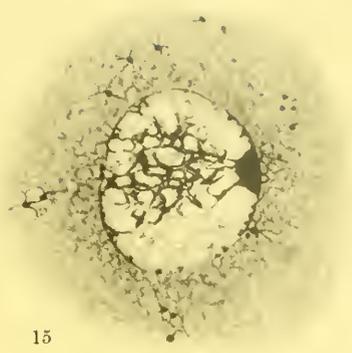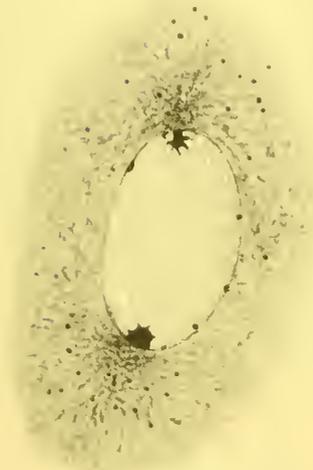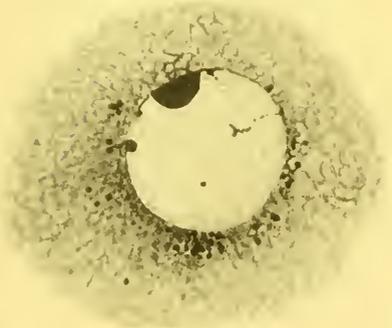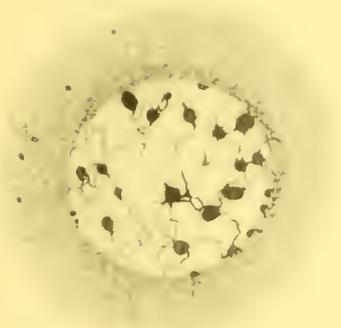
20 Nuclei of parthenogenetic eggs after remaining 25 minutes in sea-water; fragmentation of the nucleus and absorption of the chromatic substances by the small nuclei.

21 Partly fragmented nucleus developing a chromatin net.

22 Nuclei of a parthenogenetic egg after remaining 40 minutes in sea-water. Thickening of the chromatin net in the fragmented nucleus preceding differentiation of chromosomes.

23 Chromosomes of parthenogenetic egg after remaining 2 hours in sea-water. Splitting of the chromosomes without division of the nucleus.

24 Parthenogenetic egg after remaining 1 hour in sea-water. Anaphase; no chromatin granules in the cytoplasm.

25 Nucleus in metaphase of a parthenogenetic egg after remaining in sea-water 1 hour. Development of the spindle without bipolar radiation.

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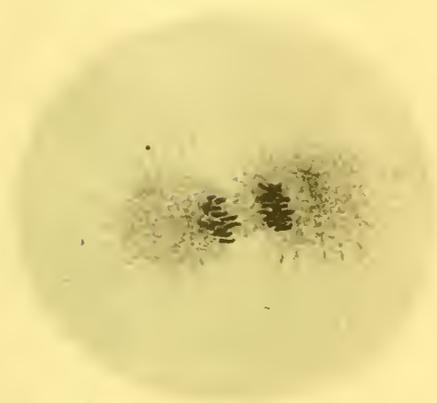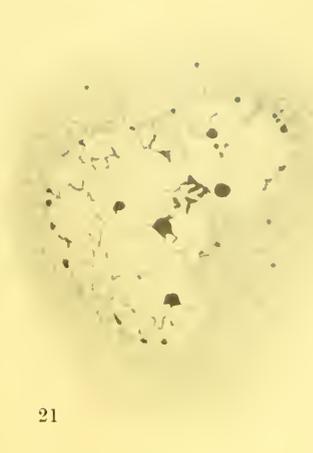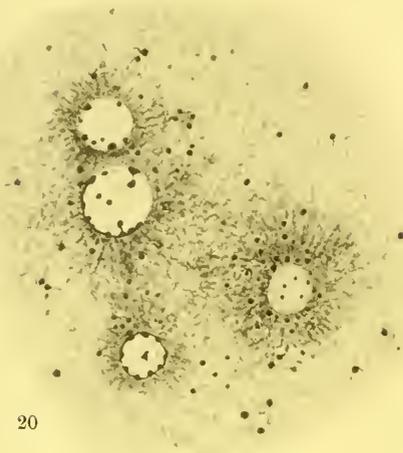
26 and 27   Egg 1 hour after fertilization.   Anaphase; spindles, chromosomes and centrosomes of a well fixed egg-figure 26. (Zenker-formol) and of a badly fixed egg-figure 27—(Zenker).

28   Nucleus of an egg 50 minutes after fertilization.   Thickening of the chromatin net before breaking into chromosomes.

29 and 30   Nuclear regions of egg 80 minutes after fertilization.   The chromosomes become vesicular and flow together.

31   Blastomere of an egg 100 minutes after fertilization.   Achromatic nucleus still showing the outlines of chromosomes; chromatic granules uniformly distributed within the nucleus between the vesicular chromosomes and in the cytoplasm.

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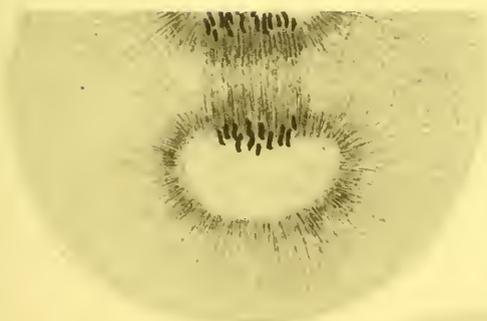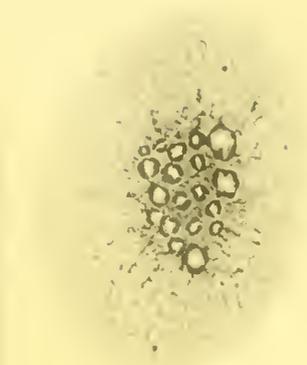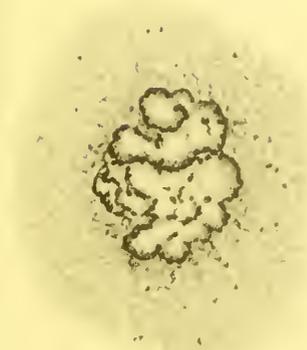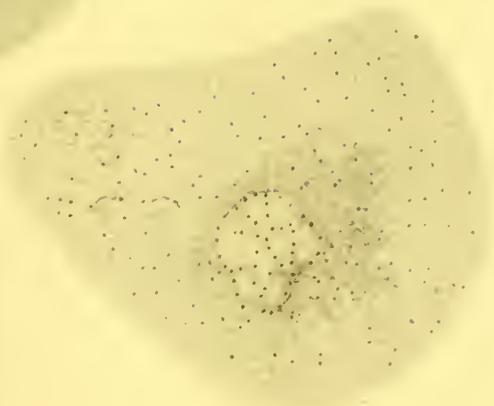
THE CHONDROCRANIUM OF A 20 MM. HUMAN EMBYRO

JOHN D. KERNAN, JR.

From the Anatomical Laboratory of Columbia University

NINE PLATES

The human chondrocrania of early stages previously described have been those of 17, 18.5 and 23 mm. embryos by Van Noorden ('87), of a 30 mm. embyro by Jacoby ('94), of 13, 14, 17, and 28 mm. embryos by Levi ('00), and of a 40 mm. embyro by Macklin ('14). As in embyros of these early periods changes by growth are exceedingly rapid, it will be seen there are intervals to be filled.

The chondrocranium here described is that of a 20 mm. human embyro, Columbia collection, no. 325, reconstructed after the method of Born, at a magnification of 75 diameters.

According to Gaupp ('00), the chondrocranium comprises four primary regions, named from behind forward, the regio occipitalis, regio otica, regio orbito-temporalis, and the regio ethmoidalis (fig. 1). The structures comprising these are grouped about a central bar of cartilage to which each is joined. This scaffold is divided into two parts, chordal and praechodral (Kolliker, '49), because of the fact that the notochord extends into the first and not into the second. The pars chordalis includes the occipital and otic regions; the pars praechordalis, the orbito-temporal and ethmoid. The two divisions are not in the same horizontal plane but form (fig. 2) an angle in the orbito-temporal region of 65°, the apex of the angle being at the corpus sphenoidale. The proximal section is called the planum basale, the distal forms the interorbital septum and the mesethmoid (fig. 1).

The planum basale forms the main part of the floor of the skull. It extends from the foramen magnum caudally[1] to the

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[1] Terms of direction are used here and throughout this article with reference to the basicranial axis.

crista transversa cranially, which marks its junction with the sphenoid body. At its caudal end it divides into two branches which join on either side the partes laterales of the occipital region. Craniad to the occipital region it has on either side the large oval masses of cartilage which make up the otic capsules. These, with the plates of cartilage which extend them dorsally form the side walls of the skull in the otic region.

Craniad to the otic region, the basal plate joins the sphenoid body. Side walls are here lacking. In their place we see two processes on each side, caudal and ventral, the alae temporales; cranial and dorsal, the alae orbitales. From the crista transversa a prominent dorsum sellae springs upward.

With the vertebral column in the vertical position, the planum as it extends ventrad, rises in level. The slope is not uniform. Caudally in the occipital region, and cranially approaching the sphenoid, there is a fairly sharp rise. Between these sloping areas is a region which is horizontal. Thus the basal plate in its sagittal extension shows two angles. The first, at the junction of the caudal inclined area and the horizontal portion, points dorsad. The second, at the junction of the ventral inclined area and horizontal portion, points ventrad. In his series of models of human chondrocrania Levi demonstrated that a change of level of the basal plate (the vertebral column being held vertical) has a great influence on the course of development of the skull. His youngest embyro (13 mm.) showed the basal plate, in so far as it was developed, horizontal and straight. A 17 mm. embyro showed a condition much like that described above for the 20 mm. a horizontal middle region joined at either end by inclined areas which form angles with it. His oldest embyro (28 mm.) showed a basal plate with almost vertical areas caudally and cranially, having a portion less steep between them. Thus the basal plate in this embryo showed the same angles as that of the earlier embryo and in addition the plate as a whole had here changed its level.

Consideration of the conditions found in all these embryos would appear to show that two processes are involved in bringing the basal plate to its final shape and level. The first is the

formation of the sloping areas cranially and dorsally, which is initiated at the 17 mm. stage and reaches its completion at the 20 mm. stage. The second process is a rotation of the whole basal plate on a transverse axis, resulting in the elevation of its cranial end. This occurs between the 20 and 28 mm. stages. The embryo reconstructed by Macklin (40 mm.) showed little change in this respect from the 28 mm. stage of Levi. According to Levi, the basal plate is formed from two anlagen, occipital and sphenoidal. These are triangular plates of cartilage arranged in such a way as to meet with apices at what will be the center of the basal plate. The base of the occipital anlage, that is the side of the triangle opposite the angle meeting the sphenoid, faces caudad and borders on the foramen magnum. The base of the sphenoidal anlage faces craniad and fuses with the sphenoid body at the crista transversa. The apices of these two triangular cartilages are joined at first only by connective tissue. In the 14 mm. stage, the cartilaginous fusion has just begun. In the 17 mm. stage, it is well established though slender. In the 20 mm. stage it is complete, and the original separation can be made out in the model only by the thinness of the cartilage. In the sections it is easily traced by the manner in which the occipital and sphenoidal regions of the basal plate form their junction, this beginning as a slender process ectally and becoming thicker as the sections are followed toward the cranial cavity, (figs. 4 and 5).

The completion of the basal plate and its fusion to the sphenoid body takes place before there is any cartilaginous otic capsule. The junction of the otic capsule to the basal plate begins in the 17 mm. stage (Levi), when the latter is already complete. This fusion is initiated cranially in the sphenoidal region, and progresses caudally. In the 40 mm. stage, Macklin could still trace a line of separation caudally by the arrangement of the cartilage cells. In the 20 mm. stage, as shown by the sections, the line of union can be traced even cranially (fig. 5, *2*). No separation of basal plate from sphenoidal anlage was found by Levi as early as the 13 mm. stage. This early fusion of the sphenoidal portion of the basal plate to sphenoid body led him

to describe the cranial portion of the basal plate as part of the orbito-temporal region. The human material at his disposal certainly permitted this interpretation. Gaupp, however, working on the lower vertebrates, has shown that the cranial portion of the basal plate is better ascribed to the otic region. All recent authors have adopted this view. (Voit '09, Mead '09, Macklin '14, DeBurlet '15).

The occipital region, (figs. 1 and 3) then consists of the occipital part of the basal plate mesially, and the paired occipital wings laterally. Cranially it joins the otic portion of the basal plate on a line with the cranial margins of the jugular foramina. The cranial surfaces of the two parts are continuous and form the clivus of the fully developed bone. Laterally the occipital wings border on the otic capsules. A line drawn parallel to the long axis of the basal plate, through the inner edges of the hypoglossal foramina, divides the mesial from the lateral parts of the occipital region.

The mesial part corresponds to the basioccipital of the human adult. It shows two distinct areas, divided by a transverse line through the cranial edges of the hypoglossa canals. The caudal area slopes sharply to foramen magnum. At this point there is a deep notch, the fore-runner of the intercondyloid notch which separates the two prominent condyles. From the notch there passes cranially on the cerebral surface a deep groove, the caudal portion of the clivus, which in the adult supports the pons Varolii. The most caudal part of this groove is in fairly close relation with the odontoid process. From the apex and cranial surface of the dens, a mass of blastema passes craniad which encloses the notochord and completely fills the groove (fig. 5, 3). The notochord passes into the cartilage on the level of the cranial edges of the hypoglossal canals and emerges ventrally and cranially just caudal to the thin area of the basal plate. Thus a large part of the basioccipital is hypochordal.

The mass of blastema filling the clivus is the anlage of the suspensory ligament of the axis.

The ectal surface of the basioccipital (fig. 3) shows an axial ridge which can be traced to the sphenoidal region. This ridge

at one point shows a prominence, the anlage of the pharyngeal tubercle of the adult (fig. 3, *28*).

The cranial area of the basioccipital is horizontal, thus making the angle with the more caudal part, which has been already mentioned in the description of the basal plate. It shows a slight concavity from side to side on the dorsal surface, and a corresponding convexity of the ventral. It gives origin on either side to the cranial root of the occipital wing (fig. 1).

The lateral parts of the occipital region may be said to take origin by two roots with the hypoglossal canal between them. The cranial entrance of this canal is divided on the left side by a thin bar of cartilage (fig. 1, *35*). This occupies only about one-third of the length of the canal, which thus appears double regarded from the ental end, but single from the ectal aspect. The two roots join lateral to the canal where they form a narrow flat plate of cartilage with surfaces facing laterad and mesiad. This plate widens quickly into the occipital wing and joins dorsally a thin plate of cartilage which surmounts the otic capsule known as the parietal plate (figs. 1 and 2).

After its junction with the occipital wing, the parietal plate narrows to a thin ribbon of cartilage which turns mesially to meet the corresponding process from the opposite side to form the tectum posterius (fig. 1, *1*). The central and lateral parts of the occipital region, in conjunction with the tectum, thus enclose the primitive foramen magnum.

The occipital wing at its origin has two borders, cranial and caudal (fig. 2). The cranial border bounds at first the jugular foramen. It is a sharp ridge and shows at one point a protuberance which Macklin takes to be the jugular tubercle of the adult. Caudal to the jugular foramen the cranial border forms the inner boundary of a space lying between the otic capsule and a lateral extension of the occipital wing known as the lamina alaris. The space is the jugular recess and in the adult condition, contains a portion of the lateral sinus (fig. 7, *3*).

The caudal border of the occipital wing bounds the foramen magnum (fig. 3). It passes from the condyle, the ventral foraminal prominence of Macklin, making a concave edge to the

dorsal foraminal prominence, a protuberance on the edge of the foramen magnum, just below the junction of the occipital wing with the parietal plate. This thick round border has been identified by Bardeen ('10), as the neural arch of the occipital vertebra.

From the lateral aspect of the cranial border of the occipital wing a prominent flange of cartilage protrudes which gives to the occipital wing a lateral extension already mentioned as the lamina alaris (figs. 2, 7, 2). The ventral tip of this structure is known as the paracondyloid process (figs. 2, 6, 1). The lamina as a whole has cranial and caudal surfaces, lateral and mesial borders, dorsal and ventral extremities. Its relation to the occipital wing on the one hand, and to the otic capsule on the other are very important. In the sections it is seen to consist of two parts, as concerns its cartilage, a lateral and a mesial (fig. 5). The mesial part is a lateral protrusion of the occipital wing (fig. 5, 5). The lateral part is a bar of cartilage parallel to the mesial ridge, and in close contact with it, but showing a separation of its cartilage at certain levels. It is entirely independent at the tip (fig. 6, 1). Then for a distance dorsal to the tip it is in close contact with the mesial part, but separated from it by a layer of perichondrium, (figs. 4 and 6, right side). Dorsal to this area of close contact, there is for a distance a fairly wide separation, then an area of complete fusion. Still more dorsally there is again an area of separation (fig. 5), then complete fusion (fig. 7, 5), which is not again interrupted.

The cranial surface of the lamina alaris forms the caudal boundary of the jugular foramen ventrally, and of the jugular recess dorsally (fig. 1, 4, 34). The caudal surface is free. The mesial edge is in relation to the outer aspect of the occipital wing as already described. The lateral edge is prominent ventrally, becomes low more dorsally, and gradually fades into the outer surface of the occipital wing. It is the inferior nuchal line of the exoccipital portion of the adult bone (fig. 2, 38).

The cranial aspect of the lateral edge of the lamina alaris (fig. 7, 5), is united to the otic capsule. The point where the two structures first come in contact marks the lateral limit of the

jugular foramen. Their cartilage is continuous for a short distance dorsal to this point, the line of union being known as the commissura occipito-capsularis (fig. 2, *33*). This line of union extends to the apex of the otic capsule, where there is a break in the cartilage known as the fissura capsulo-parietalis. The interruption found by Macklin in his 40 mm. embryo and designated by him fissura capsulo-occipitalis is also present in this embyro.

From the tip of the paracondyloid process there extends mesaid and craniad (figs. 2, 3, 4, 5), a thin process of cartilage which reaches the basioccipital, passing laterad and craniad to the hypoglossal canal. This was found by Macklin in his 40 mm. embryo, where it bounded laterally and cranially a foramen which he termed paracondyloid foramen. It corresponds in position to a ridge of bone which in the adult passes from the tip of the jugular process, mesiad and craniad to basioccipital. It represents, as Macklin suggests, a costal process, not that of the occipital vertebra, but of one of the unsegmented vertebrae since its connections to the basioccipital and occipital wing are craniad and laterad to the hypoglossal foramen.

The paracondyloid process was described by Levi in all the embryos examined by him and figured prominently in his models. He interpreted it to be the tip of the neural arch of the occipital vertebra. Macklin has sufficiently demonstrated the erroneousness of this view, and considers it to represent the transverse process and possibly the costal process of that vertebra.

As has been pointed out the paracondyloid process is but the free extremity of the lamina alaris, and this has been shown to be a bar of cartilage uniting at intervals with a parallel ridge of the occipital. It is therefore suggested that in the lamina alaris are represented the costal and transverse processes of several vertebrae. The outer bar represents the fused costal elements and may be termed the costal bar. The points of union correspond to the bases of the transverse processes, the areas of separation indicate the intervals between the processes of successive vertebrae. If this interpretation prove correct, three such processes can be enumerated.

That of the occipital vertebra is small and shows no independence of the costal element. It will be noted that this transverse process, although in close contact with the costal bar, has not yet formed a cartilaginous union with it (fig. 4, 6). This would indicate that the costal and transverse processes of the occipital vertebra take only a minor part in the formation of the lamina alaris and paracondyloid processes. The second vertebra shows an independent costal element which is drawn out caudad to form the paracondyloid process. From its tip there passes to the basioccipital that bar of cartilage already described as a rib process. It is because of the connection of the paracondyloid process to the basioccipital and occipital wing craniad and laterad to the hypoglassal canal that it is interpreted as the rib process of the second occipital vertebra. The most cranial vertebra helping to form the basioccipital shows no free costal element. The costal bar is prolonged upward on the occipital wing as a ridge which represents, as already said, the inferior nuchal line of the adult bone.

That the occipital region of mammals is made up of a number of vertebrae, all authors agree. The exact number has not been definitely decided. Froriep ('86) found four in calf embryos. Mead states the number as being three.

As far as this present embryo offers any evidence on this point at all, the number would appear to be three, that number of transverse processes and ribs being indicated.

Levi analyzed the human occipital region as follows: The condyles he considered to represent the ununited body of the occipital vertebra; the area between, reaching craniad to the primitive break in the basal plate, he considered as the result of the fusion of the body elements of the unsegmented vertebrae. The paracondyloid process he called the tip of the arch of the occipital vertebra; and the cartilage craniad to the hypoglossal foramen, the fused neural arches of the unsegmented vertebrae. He did not account for the cartilage bordering the foramen magnum dorsal to the condyles.

Macklin considered that the body element of the occipital vertebra also helped form the area between the condyles border-

ing on the foramen magnum.   He represented everything mesiad
to a line drawn from the cranial tip of the jugular foramen to the
ventral foraminal prominence as containing body elements.
The outer part of the condyle he took to be the inferior articular
process, the jugular tubercle the superior articular process; the
paracondyloid process, the transverse and costal elements; and
the thick area bordering the foramen magnum, the neural arch, all
belonging to the occipital vertebrae.   The neural arch elements
of the unsegmented vertebrae he said fused with that of the
occipital vertebra, to form the occipital wing, and their pedicles
all fused into the comparatively small bar of cartilage cranial to
the hypoglossal canal.

There are some objections to this analysis.   The jugular
tubercle belongs to the structures craniad of the hypoglossal
canal.   Hence it cannot very well be the superior articular
process of the occipital vertebra (fig. 1).

The condyle (fig. 2) cannot be an inferior articular process as
found in the cervical vertebrae, since its relations to surrounding
structures are fundamentally different.   Thus, the condyle is
ventral to the transverse process of the occipital vertebra, ven-
tral to the transverse plane through the notochord, and ventral
to the suboccipital nerve, whereas the articular processes of the
cervical vertebrae are dorsal to these structures (fig. 2).

The condyle has the same relations to the transverse and costal
processes of the occipital vertebra and to the chorda as has the
lateral mass of the atlas to its transverse and costal processes. As
shown by H. Gadow ('96), the atlas may be analyzed as follows:
the lateral mass is formed by the fusion of basiventral and basi-
dorsal elements where they meet at the side of the chorda.   The
basiventral elements, here retained, form the ventral arch of the
atlas. The basidorsal elements, form the neural arch of the atlas.
The centrum fuses with that of the axis to form the dens epis-
trophei.

It is possible that the occipital vertebrae are vertebrae of the
atlas type.   Then the condyles and the tissue immediately
dorsal would be formed by fusion at the junction of basiventral
and basidorsal elements; the hypochordal elements bordering the

intercondyloid notch mentioned in describing the course of the notochord, would be basiventrals, and the occipital arch and wing would be fused basidorsals. The centra would be found in the mass of connective tissue which surrounds the chorda and which eventually forms the suspensory ligament of the odontoid process (figs. 4 and 5). The portion of basioccipital dorsal to the chorda remains to be accounted for. Examination of the occipital region of early cat embryos shows that the condylar portions and the hypochordal cartilage joining them ventral to the chorda are formed before there is any suprachordal cartilage. The chorda lies in a dorsal groove and extends craniad without dorsal cartilaginous covering. In later stages cartilage forms about the chorda and covers the cranial portion of the groove in the basioccipital. It is this perichordal element which forms the suprachordal cartilage of the basioccipital and its cranial extension, the clivus. It does not belong to the occipital vertebrae, and its fusion to the occipital region is only owing to the intimate relations of the chorda to both elements.

There is some evidence to support this view of the occipital vertebrae. First, the fact that in some lower animals (Talpa, E. Fischer '01), in early stages the atlanto-occipital joint is horse-shoe-shaped, partially surrounding the foramen magnum anteriorly. This articulation may better be interpreted as a joint between the two anterior arches, such as occurs in birds between anterior arches of atlas and axis, (it persists partly in this latter vertebra), than as an articulation between vertebral body and anterior arch, which it would be were the anterior edge of the foramen to consist of body element.

Second, as I have already pointed out, the relation of transverse and costal elements are the same in respect to the lateral mass of the atlas and to the condyle of the occipital cartilage (fig. 2).

Third, the manner of chondrification is much the same. As described by Levi, it begins in the occipital cartilage, (figs. 8, 9, B), laterally, just mesial to the hypoglossal canal. These centers unite ventrally, and the chondrification proceeds dorsally, gradually obliterating the groove between the lateral foci. This

manner of chondrification explains the appearance of the model at this stage, the thick condyles laterally, united ventrally in a ridge but separated dorsally by a deep groove (fig. 6).

The atlas similarly begins to chondrify in its lateral masses, which later unite ventrally to form the anterior arch (figs. 8 and 9, *B*).

In calf embryos Froriep found absorption of the hypochordal arches, the basiventrals, and interpreted the median region of the occipital as derived from the centra of occipital vertebrae. DeBurlet, in Balaenoptera rostrata, has shown the caudal end of the occipital region to be suprachordal in position, not hypochordal, a condition which plainly excludes the possibility of homologizing this region with basiventrals.   On the other hand Weiss ('01), working with rat embryos, Noordenbos ('05), with Talpa, and Gaupp with rabbit and opossum embryos, have all shown that in these animals the basioccipital is formed by hypochordal elements.   It is evident that we are in need of further investigation of this region.

My embryo shows the oldest cartilage just mesial to the hypoglossal canals, and in the arches close to the outer side of the canals.   There is no sign of bone formation.

The occipital wing shows on its outer surface, lateral to the edge of the foramen magnum, the thin area of cartilage known as the paraforminal area (Macklin) (figs. 2, 3).   There is no sign of the lateral occipital protuberance.   On the ental surface the groove between occipital wing and parietal plate, the occipito-parietal groove (Macklin) may be traced, but there is no occipito-parietal fissure (fig. 1).   The internal capsulo-occipital groove is well marked; the external, only fairly well (figs. 1, 2, 3).

The otic region is composed of the otic portion of the basal plate, the paired otic capsules, and the paired parietal plates.

The otic portion of the basal plate (figs. 1 and 3) is thick and wide caudally where it joins the occipital region.   This juncture is caudal to the site of that primitive break in the cartilage already described.   The plate here is horizontal.   Cranially the dorsal surface rises rather sharply, becomes convex, and passes without demarcation into the steeply sloping dorso-caudal sur-

face of the dorsum sellae. The ventral surface of the otic portion of the basal plate (fig. 3), craniad to the primitive break, becomes convex from side to side and continues its horizontal direction. This divergence of the surfaces causes a dorso-ventral thickening of the cartilage which is much more marked further craniad in the orbito-temporal region (fig. 2). The line of demarcation between otic and orbito-temporal regions may be placed at the beginning of the rise of the surface to the dorsum sellae.

The otic capsules are firmly fused to each side of the otic portion of the basal plate, which they deeply indent and so narrow. Of the character of the fusion, I have already spoken (fig. 5). The line of fusion is marked dorsally and ventrally by deep grooves known as the basi-capsular grooves (DeBurlet). These grooves together completely encircle the line of union and meet cranially and caudally in notches, the spheno-capsular and occipito-capsular respectively (figs. 1 and 3). The first is the primitive carotid canal, the second is the ventral end of the jugular foramen.

The capsules have two other connections to surrounding parts. The first is caudally through the occipito-capsular commissure to the lamina alaris of the lateral occipital region; the second is dorsally through the capsulo-parietal commissure to the parietal plate (figs. 2 and 7).

The otic capsule consists of two parts, (fig. 1) the cochlear, which contains the vestibule and cochlea; and the canalicular, which contains the semicircular canals. While the cartilage of these regions is continuous and the capsule appears in a single mass in reconstruction, on microscopic examination the cochlear part is found to be much younger, an evidence of the retardation of its chondrification, which is the usual condition in mammals. The union between the two is marked cranially and caudally by notches which correspond to the superior and inferior otic notches of Macklin (figs. 1 and 3). Mesially, the surfaces of the two parts pass smoothly into one another without demarcation. The line of union seen from below (fig. 3), is a deep groove in which the facial nerve passes forward to enter the skull by turning dorsad through the superior otic notch. The ventral sur-

face of the pars canalicularis joins the lateral surface of the pars cochlearis at an angle to form this facial groove which lies at the bottom of the ventro-lateral otic recess of Macklin. This contains the anlagen of the otic ossicles.

The cochlear portion is ventral, cranial and mesial to the canalicular portion (figs. 1 and 3). It is oval, the long axis pointing cranially and mesially. Lateral, mesial and dorso-caudal surfaces are distinguished.

The lateral and mesial surfaces are divided by a rounded border which passes from the peri-lymphatic foramen below, around the cranial pole, to the superior otic notch above. The first part of this border deviates outward to form the promontorium (fig. 3, 29). It divides the peri-lymphatic foramen from the region of the foramen ovale. The lateral surface is smooth and convex. Dorsally, at the junction with the ventral surface of the canalicular portion, is the facial groove. The outlines of the fenestra vestibuli cannot yet be made out, as the foot plate of the stapes is buried in indifferent tissue (fig. 3). There is trace of the sulcus caroticus found by Macklin in the 40 mm. stage in the sections (fig. 4, 20).

The dorso-caudal surface is taken up by a large opening, the perilymphatic foramen (fig. 3), which includes the future fenestra cochlearis and the ductus perilymphaticus. The division of these two by a processus interperilymphaticus, well marked in the 40 mm. stage of Macklin, has here scarcely begun (fig. 3, 11), The outer edge of the common opening juts laterad to form the promontory.

The mesial surface is divided by its junction with the basal plate into ventral and dorsal sections. The ventral is smooth, convex, and forms the external wall of the ventral basi-capsular fissure (fig. 3, 9). Above the union with the basal plate the surface is smooth, convex, and continuous dorsally with the surface of the canalicular portion. Cranially the opening of the internal acustic foramen appears (fig. 1). This is rather wide open cranially and mesially. This condition is what we would be led to expect from an examination of the models of Levi. In his model of the 17 mm. chondrocranium, the internal acustic

foramen gaps widely ventrally, mesially and cranially. Evidently, as comparison with my model shows, enclosure of this foramen by cartilage begins caudally and laterally and proceeds craniad and mesiad. This holds true for the whole cochlear portion, as in Levi's 14 mm. model there is no cartilage about the cochlea except caudally and laterally.

The facial nerve (figs. 1 and 2) is directed laterad and craniad from the internal acustic foramen to the cavum supracochleare (Voit), passing between the pars cochlearis and pars canalicularis. It then turns sharply caudad, ventral to the bar of cartilage which unites the pars cochlearis with the pars canalicularis. At this angle where its direction changes, it presents the enlargement of the geniculate ganglion and gives off the great superficial petrosal nerve. The portion of the nerve proximal to the ganglion transverses the primitive facial canal, which has as yet no cartilaginous roof. Conditions here, in view of the theoretical importance of the region, must be discussed in some detail. At the site of the future mesial commissura suprafacialis—this Voit holds to be equivalent to the commissura praefacialis of reptiles—the pars canalicularis sends a small projection mesiad on the dorsal aspect of the nerve (fig. 1, *31*). This is directed towards the pars cochlearis, but is far from forming a complete bridge. The commissura is well developed at 40 mm. (Macklin, fig. 6), and at 80 mm. it forms a broad unperforated roof to the facial canal (Hertwig, fig. 658). Fischer, however, states that in a special model by Hertwig of the otic region of this same human embryo, the roof of the facial canal is perforated. In this he finds evidence of the existence of two suprafacial commissures in man, which he previously had ascertained to be the case in Talpa, a medial and a lateral, one crossing the facial nerve proximal, the other distal to the geniculate ganglion. Of this second commissure there is no trace in this embryo, nor does Macklin record its presence at 40 mm. Its very late appearance in man would suggest the possibility of its being of secondary nature, and in so far supports Voit's interpretation of the mesial commissure as the fundamentally important one and equivalent to the commissura praefacialis of rep-

tiles.   It would be desirable to have further information regarding the history of the lateral bar in man, both as regards its constancy and the details of its formation, before accepting as final its interpretation as part of the primitive praefacialis commissure.

In this connection, Voit's observations on the rabbit are of importance.   Here the lateral commissura praefacialis is connected plainly with the tegmen tympani and joins the pars cochlearis distal to the geniculate ganglion and, in particular, ventral to the great superficial petrosal nerve.   This process is represented in my embryo by a triangular flange of cartilage projecting from the pars canalicularis in the angle of the external genu of the facial nerve (fig. 3, 5).   From its position it can, in the embryo, be inferred to be equally capable of reaching the pars cochlearis on either the dorsal or the ventral aspect of the great superficial petrosal nerve.

The pars canalicularis is a three-sided pyramid with its base ventral, apex dorsal, on the dorsal aspect of the pars cochlearis. The surfaces are lateral, cranio-mesial, caudo-mesial.   Each border of the triangular outer surface marks the course of one of the semicircular canals, to wit: the anterior border that of the anterior vertical canal, the posterior border that of the posterior vertical canal, the inferior border that of the external canal (fig. 2).   The apex corresponds to the highest point in the arch of the anterior vertical canal.   The external canal makes a well defined prominence on the outer surface.   The course of the others is less clearly indicated in the surface relief.

The cranio-mesial surface is framed by the arch of the anterior vertical canal.   Beneath this is a well marked fossa subarcuata. This surface is bounded caudally by a ridge formed by the union of anterior and posterior semicircular canals, the crus communis. This ridge is the dividing line between the two mesial surfaces. Upon this ridge is the opening of the ductus endolymphaticus (fig. 1, 10).

The inferior edge of the external surface is extended downward to bound the outer side of the facial groove.   It presents posteriorly a small mastoid process.   Cranial to the mastoid process is a prominent ridge, the crista parotica.   At the cranial end of

this inferior border of the external surface, a slender process of cartilage juts forward which Macklin interprets as the anlage of the tegmen tympani (fig. 2, 5).

The base of the pyramid is ventral (fig. 3). It is roughly triangular in shape, with the apex of the triangle covered by the pars cochlearis. The external border is marked by the series of processes already described, to wit: the mastoid process, the crista parotica, the tegmen tympani. To the middle of this border is attached the short process of the incus. At the dorsal extremity of the crista parotica, Reichert's cartilage is received in a depression of the cartilage (fig. 5, 18).

The parietal plate (fig. 2, 1) is a thin sheet of cartilage which surmounts the pars canalicularis and is continuous with it. Ventrally this ends in a pointed process overhanging the capsule. Dorsally it continues as a thin ribbon of cartilage, turns inwards and meets the process from the opposite side. Thus is enclosed the primitive foramen magnum.

The ventral borders of these parietal plates are continuous with the occipital wings, though the line of union is marked by a groove on the inner surface, the occipito-parietal groove (fig. 1, 2). Thus they seem in this embryo to have a more intimate union with the otic capsules than with the occipital wings, and they may be considered as forming a true tectum synoticum. In the 40 mm. stage Macklin found a more ventrally placed union of the occipital squama, (the tectum posterius). Above this union he found the parietal plates to end with free dorsal edges. Levi showed that the dorsal union between the two sides occurred cranially and advanced caudally and ventrally, the more cranial union between the parietal plates being absorbed as the ventral union is formed. The conditions in Macklin's 40 mm. and this 20 mm. embryo would appear to bear out this statement of Levi.

The significance of the mode of union of the two sides would seem to be that the tectum synoticum is a more primitive structure and is so formed early, and is absorbed as the tectum posterium reaches its development.

Jacoby was the first to point out that the parietal plates are temporary structures which are early absorbed. Indeed, in the

14 mm. embryo of Levi they appear larger proportionately than in this 20 mm. model, and they show successive decrease through his series. We may also conclude that the dorsal extensions of the parietal plates which result in the formation of the tectum synoticum share in the temporary nature of the rest of the structure.

In my description of the otic capsule, I have placed the model with the vertebral column vertical and the basal plate horizontal. After the rotation of the basal plate takes place, the capsule being carried with it, the ventral pole becomes cranial, the cranial pole dorsal. This occurs between the 20 mm. and 28 mm. stages. The change accounts for my using different terms of position from those of Macklin for the same points.

The orbito-temporal region consists of the sphenoid body mesially, and two processes on each side, the alae temporales and the alae orbitales (fig. 1).

I have already described the manner in which the planum basale thickens as it approaches this region. From the crista transversa the prominent dorsum sellae projects cranially and dorsally (fig. 2, 7). This appears to be firmly united to the crista transversa by continuous chondrification, not separated as described by Fawcett ('10), in the 19 and 21 mm. stages. Cranial to this ridge the surface is hollowed by a transverse gutter, the sella turcica (figs. 1 and 2, 8). The floor of the sella turcica is formed by the expanded cranial end of the basal plate. Cranial to the sella, the surface rises dorso-craniad to form a limiting wall of the sella in this direction (fig. 1). This wall should be noted, as Levi showed that in the 17 mm. stage, the sella had no wall cranially, making one surface with the lamina hypochiasmatica. The lamina hypochiasmatica (figs. 1 and 2) is a broad flat surface in front of the sella turcica, on a different level. This change of level Levi found established in his 28 mm. embryo, the next oldest in his series. It is however perfectly developed in this embryo of 20 mm. Fawcett does not describe it. Levi makes the point that the floor of the sella retains its original position when the planum basale heaves up and the 'amina hypochiasmatica is elevated. It is interesting to note

that the latter change is pretty well completed when the former
has only just begun. The caudo-dorsal edge of the lamina hypo-
chiasmatica is the forerunner of the tuberculum sellae. There
are no traces of the middle clinoid processes. The plane of the
floor of the sella turcica forms an angle of 115° with the upper
surface of the planum basale. It is from the angle formed by
their junction that the dorsum sellae rises.

The lamina hypochiasmatica is limited cranially by a trian-
gular projection dorsad of the caudal extremity of the meseth-
moid. This will be described in connection with the ethmoid
region (figs. 1 and 8, 6).

At the lateral edge of the lamina hypochiasmatica there are
paired little cylinders of cartilage which have their long axes
parallel to the plane of its surface (figs. 1 and 3). These are
known as the alae hypochiasmaticae (Voit). They are very well
developed in the rabbit. Macklin found them well developed
in the 40 mm. stage of the human and mentions them as having
never been described in man. Fawcett ('10), however, had fig-
ured them in the illustrations of his article on the development
of the human sphenoid bone, but described them as being con-
tinuous with the alae orbitales. The sections show in this em-
bryo (figs. 4, 5, 6) that they are independent of both ala orbitalis
and lamina hypochiasmatica, though they unite later with the
dorsal roots of the former (Fawcett) and the sides of the latter
(Macklin).

The alae orbitales (figs. 1 and 3) have as yet no connection to
the sphenoid. They are S-shaped plates of cartilage which ex-
tend craniad and dorsad from the caudal end of the alae hypo-
chiasmaticae. Neither root is as yet formed, though there are
indications of the caudal root mesial to the caudal ends of the
alae hypochiasmaticae. There are as yet no indications of the
ventral, lateral and dorsal extensions found by Jacoby at 30 mm.
and Levi at 28 mm., which later unite the alae to the inter-or-
bital septum, to the ectethmoid, completing the optic foramen
and forming the roof of the orbit. Also the dorso-lateral exten-
sion, mentioned by Jacoby and Macklin as extending toward
the parietal plate and representing the commissura orbito-
parietalis of other mammals, is not present.

The alae temporales (figs. 1 and 3) are caudo-ventral to the alae orbitales. In them two parts are distinguished, mesial and lateral (Fuchs '10) (figs. 4, 5, 6). The mesial part, cranially is known as the processus alaris. It is a short bar of cartilage which arises from the ventro-cranial edge of the sphenoid body and extends caudo-laterad. From the point of origin of the lateral part of the temporal wing it continues caudally as the processus ali-cochlearis to the cochlea, with which it comes in contact, but does not unite. This whole process was described by Jacoby as a cartilaginous bridge from the sphenoid to cochlea, from the outer edge of which arose the outer part of the ala temporalis or lamina ascendens. Levi found no trace of it in his models. Macklin described it as approaching the cochlea, but not very closely.

In the region where it might be expected to join the cochlea, he found a separate nodule of cartilage which he called the supra-cochlear cartilage. The dorsal division, the commissura ali-cochlearis, is not seen in Hertwig's models. It would appear to be only a temporary structure in man, though found in many mammals (DeBurlet, Olmstead '11, Voit, Fischer). When present, it bounds the carotid canal laterally.

The lateral part of the ala temporalis (figs. 1, 4 and 6), or the lamina ascendens, is a rhomboidal block of cartilage attached by its mesial, dorsal corner to the processus alaris. Its ventral edge is narrow; the dorsal edge is broadened into a flat surface which the foramen rotundum perforates downward and forward (fig. 6). The long axis of the structure points laterad. From its caudal edge arise two processes of dense connective tissue which embrace the third division of the fifth nerve, which is just caudal to the ala temporalis. Thus is indicated the beginning of the foramen ovale (fig. 4).

There is no indication in the orbito-temporal region of any remains of the primitive side wall mesial to the Gasserian ganglion and the structures contained in the cavum epiptericum and cavum supra-cochleare (Voit), such as Voit found in the rabbit. In his 40 mm. reconstruction, Macklin thought this primitive wall might be indicated by the isolated nodule of cartilage at the

cranial pole of the cochlea, which he called the supra-cochlear cartilage. DeBurlet, in his examination of the primordial cranium of the whale, demonstrated that the commissura ali-cochlearis, to which that nodule probably belonged, was outside the primitive skull. Examination of the sections show the beginning of the formation of the side wall lateral to the Gasserian ganglion in the form of a dense blastema which passes from the outer side of the pars canalicularis craniad a variable distance to the outer side of the skull vault. In such of the lower mammals as I have examined (cat, opossum, Tupaia) this process is cartilage, and in them forms the parietal plate dorsally and the commissura orbito-temporalis ventrally, which is homologous to the taenia marginata of reptiles (figs. 5 and 7).

As concerns its cartilage, the ethmoid region is very rudimentary. The mesethmoid consists of a mesially placed plate of cartilage which begins dorsally as a triangular bar with its base against the cranial surface of the sphenoid body, and its apex projecting upward in front of the lamina hypochiasmatica (figs. 1, 2 and 7). In this region, as Macklin points out, it forms the inter-orbital septum, homologous to the inter-orbital septum of reptiles. As it extends forward, the base of the triangle narrows, and distally it forms a flat nasal septum. This gradual flattening can be followed in the sections. At the cranial extremity, the dorsal edge protrudes upward to form the anlage of the crista galli (fig. 2). From the ventral edge of the septum project two slender ridges of pre-cartilaginous tissue which are the anlages of Jacobson's cartilages (fig. 9, A). They are not connected with the cartilage of the septum, but appear to be in a sheet of dense blastema which extends dorsally on each side of the septum (fig. 3).

The ethmoid region presents laterally two small flat plates of cartilage with their dorsal edges inclining toward the septum (fig. 9, A). There is only slight indication as yet of cartilaginous turbinates.

Rudimentary as is the cartilage, the nasal cavities as laid down in pre-cartilage are much more complete (fig. 9, A) as shown by examination of the sections, in which septum and side walls can

be followed from nasal tip to sphenoid body. The connective tissue mentioned by Fawcett as covering in the nasal region at the site of the future nasal bones and the frontal is present.

Lateral and ventral to the nasal region, the maxillae are beginning to form. As Fawcett states, there is one center of ossification. Frontal, palatal, and alveolar processes can be identified (fig. 9, *A*).

In the course of the description of my own reconstruction, I have dealt with the differences as compared to the reconstructions of Levi and Macklin.

Von Noorden reconstructed 18.5 mm. and 23 mm. embryos. It is to be regretted that his descriptions and illustrations are not very complete. As compared to the 18.5 mm. embryo described by him, the following advances may be noted in the 20 mm. stage:

1. Formation of foramen rotundum, and outline of foramen ovale. 2. Formation of carotid canal by the extension of the commissura ali-cochlearis to the ventral pole of the cochlea. 3. Appearance of mastoid processes. 4. Joining of the parietal plates to form the tectum posterius. 5. Appearance of maxillae.

Von Noorden states that the optic foramina are formed in his embryo. There is no indication of them in mine.

As compared to Jacoby's 30 mm. embryo, my reconstruction shows (1) much less development of the nasal region. In Jacoby's reconstruction there are well-developed side-walls which are connected with the septum to form the nasal roof. (2) Lack of cartilaginous connection between alae orbitales and pre-sphenoid and ethmoid regions. Examination of Jacoby's model shows that the alae orbitales have three connections to neighboring cartilaginous structures, to wit: to the pre-sphenoid through the taenia post-optica, to the interorbital septum through the pro-optica, and to the ect-ethmoid through the cartilago spheno-ethmoidalis. All three of these processes are undeveloped in my embryo. This accounts for the absence of the optic foramen and the fissura orbito-nasalis. Another extension of the ala orbitalis, dorsal and caudal toward the parietal plate, present in Jacoby's embryo, is absent in mine. Briefly, at this stage all the

cranial, lateral and caudal parts of the orbital wings have not
begun to develop. (3) Almost the entire absence of the turbi-
nates, which Jacoby finds beginning to form. Levi mentions
them as being already cartilaginous in his 28 mm. embryo. (4)
There are, in my embryo, no external pterygoid processes, which
Jacoby found in his.

In the sphenoid region, my embryo seems, in certain respects,
more developed, as it shows a posterior clinoid process, a foramen
rotundum, and incomplete foramen ovale.

As compared to the fully developed chondrocranium, my
model lacks (1) in the nasal region, the nasal roof, the lamina
cribrosa, the connection between ala orbitalis and sphenoid,
and the turbinates. (2) In the sphenoid region there is no
optic foramen; the alae orbitales are poorly developed and are
not connected with the sphenoid body; the pterygoid is lacking.
(3) In the otic region, the tegmen tympani has only just begun
to form. (4) In the occipital region, the disproportionate size
of the foramen magnum at once attracts attention, due to the
occipital wings not having met in the midline. The paracondy-
loid processes are disproportionately large; in later stages they
undergo absorption. The fact that they are not yet entirely
fused with the occipital squama should be particularly noted.

As to the light which this reconstruction throws on the course
of the development of the skull, this may be said: The basal plate
first fully develops. The occipital squamae develop and unite
about the foramen magnum, first through the slender band of
cartilage which connects the partes caniculares and fuses with
the squama by its caudal edge.

The development of the otic capsules and sphenoid body takes
place about the same time, and the otic capsules then fuse to
basal plate and occipital squamae. The orbital and temporal
wings of the sphenoid region next appear, and a little after them
the nasal septum and lateral nasal parts. This is about the
stage of my embryo.

The next step (Levi) is the elevation of the ventral end of the
basal plate, involving rotation also of the otic capsules through
almost one-quarter of a circle. This causes the ventral ends of

basal plate and otic capsules to take a dorsal position. The nasal region, orbital wings, are now developed and ossification sets in, first in the maxillae and mandibles. From the 20 mm. stage to the 40, the brain cavity expands laterally becoming wide and shallow instead of narrow and deep.

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FIGURE 1

Dorsal view of chondrocranium, 20 mm. human embryo.

1, Tectum posterius
2, Sulcus occipito-parietalis
3, Sinus lateralis
4, Foramen jugulare (Occipito-capsular notch)
5, Foramen hypoglossi
6, Dens epistrophei
7, Chorda dorsalis
8, Planum parietale
9, Fissura parieto-capsularis
10, Foramen endolymphaticum
11, Nervus facialis
12, Ganglion geniculatum
13, Sulcus basicapsularis dorsalis
14, Nervus petrosus superficialis major
15, Foramen ovale
16, Foramen rotundum
17, Foramen caroticum (Spheno-capsular notch
18, Dorsum sellae
19, Septum interorbitale
20, Maxillo-turbinal
21, Crista galli
22, Septum nasi
23, Paries nasi
24, Lamina hypochiasmatica
25, Ala hypochiasmatica
26, Ala orbitalis
27, Processus alaris
28, Ala temporalis
29, Processus alicochlearis
30, Porus acusticus internus
31, Processus praefacialis
32, Fossa subarcuata
33, Ridge of crus communis
34, Recessus jugularis
35, Bar dividing foramen hypoglossi
36, Cranial root of occipital plate
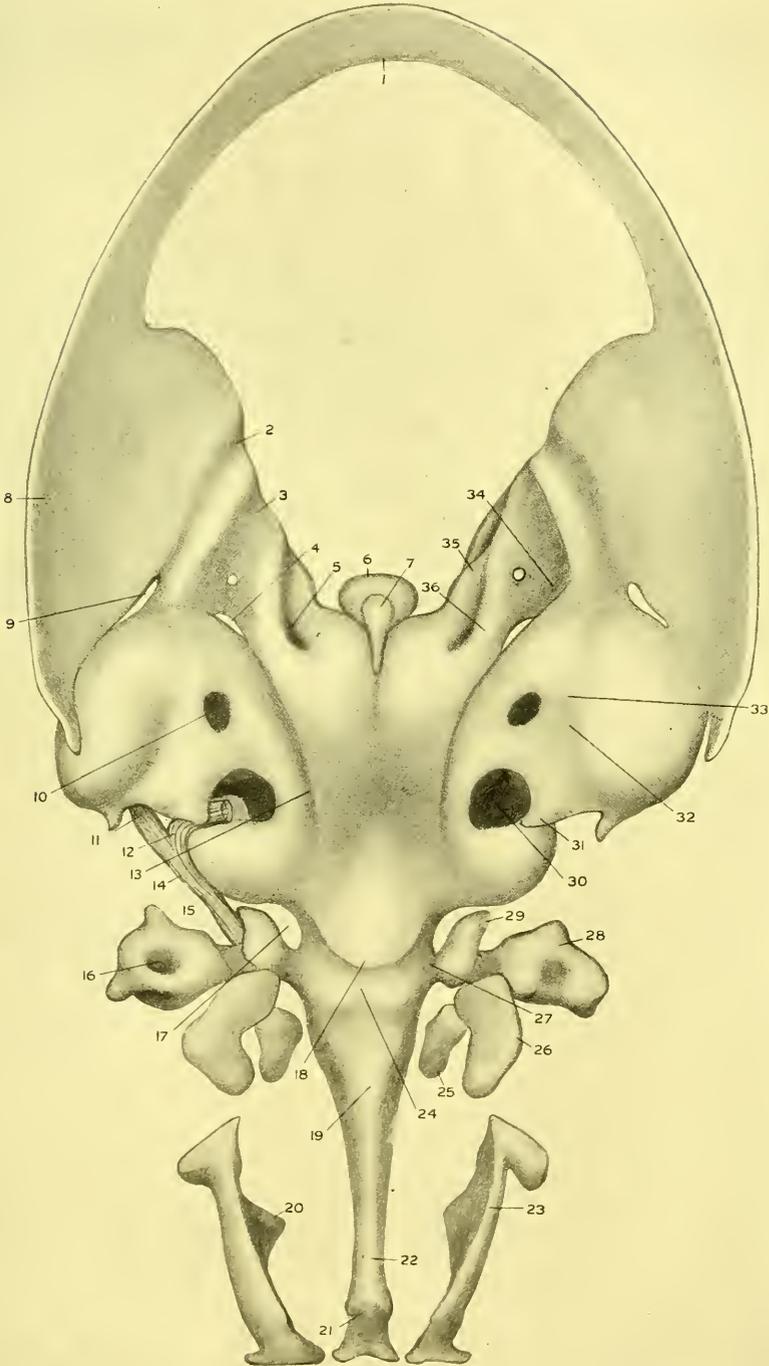
FIGURE 2

Right side of chondrocranium and anterior cervical vertebrae of a 20 mm. human embryo.

1, Planum parietale
2, Fissura parieto-capsularis
3, Canalis semicircularis superior
4, Stapes
5, Tegmen tympani
6, Pars cochlearis
7, Dorsum sellae
8, Sella turcica
9, Ala orbitalis
10, Ala hypochiasmatica
11, Paries nasi
12, Maxilla
13, Anlage of cartilago paraseptalis
14, Septum nasi
15, Ala temporalis
16, Processus alicochlearis
17, Meckel's cartilage
18, Mandibula
19, Manubrium mallei
20, Incus

21, Processus longus incudis
22, Chorda tympani
23, N. facialis
24, Reichert's cartilage
25, Canalis semicircularis inferior
26, Costal process 2nd occipital verte bra
27, Arcus anterior atlantis
28, Processus mastoideus
29, Dens epistrophei
30, Processus costalis atlantis
31, Processus transversus atlantis
32, Processus paracondyloideus
33, Commissura occipito-capsularis
34, Arcus atlantis posterior
35, Costal bar, Lamina alaris
36, Paraforminal area
37, Fissura occipito-capsularis
38, Crescentic ridge
39, Tectum posterius
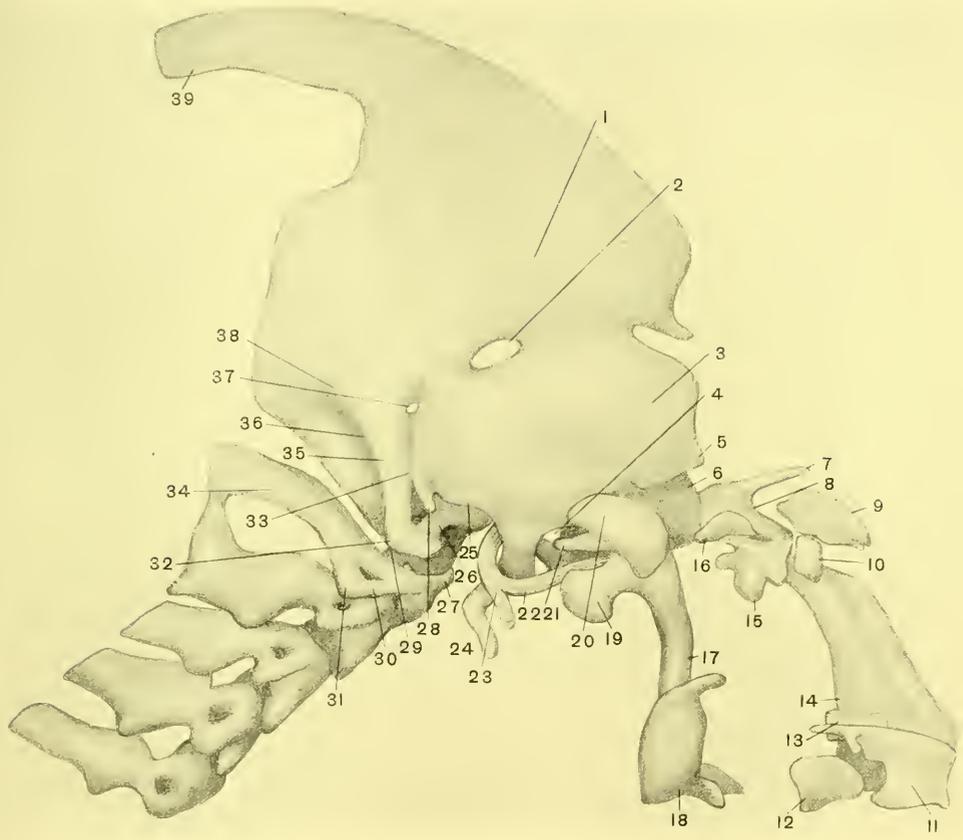
FIGURE 3

Ventral view of chondrocranium of a 20 mm. human embryo.

1, Paries nasi
2, Maxillo turbinal
3, Anlage of cartilago paraseptalis
4, Ala hypochiasmatica
5, Ala orbitalis
6, Processus alaris
7, Ala temporalis
8, Processus ali cochlearis
9, Sulcus basicapsularis ventralis
10, Tegmen tympani
11, Processus interperilymphaticus
12, Sulcus facialis
13, Stapes
14, Crista parotica
15, Foramen perilymphaticum
16, Processus paracondyloideus
17, Processus mastoideus
18, Prominentia foraminalis posterior

19, Processus costalis et transversus, occipital vertebra
20, Foramen jugulare
21, Prominentia foraminalis anterior (condylus)
22, Intercondyloid notch
23, Hyale
24, Processus longus incudis
25, Incus
26, Malleus
27, Maxilla
28, Pharyngeal tubercle
29, Thin area in basal plate
30, Promontorium
31, Rib element, 2nd occipital vertebra
32, Chorda dorsalis
33, Hypophyseal stalk

FIGURE 4

Section of 20 mm. human embryo, nearly parallel to basal plate.

1, Slender area of basal plate
2, Blastemal anlage of ligamentum dentis epistrophei
3, Costal bar of lamina alaris
4, Transverse and costal process, occipital vertebra
5, Cranio-mesial extension of costa bar
6, Perichondrial layer separating costal bar and transverse process of occipital vertebra
7, Ala hypochiasmatica
8, Ala temporalis, processus alaris
9, Ala temporalis, processus ascendens
10, Foramen rotundum
11, Foramen ovale
12, Processus alicochlearis
13, Chorda dorsalis
14, Hypophyseal stalk
15, Malleus
16, Incus
17, Stapes
18, Reichert's cartilage
19, Interhyale
20, Arteria carotis
21, Foramen perilymphaticum
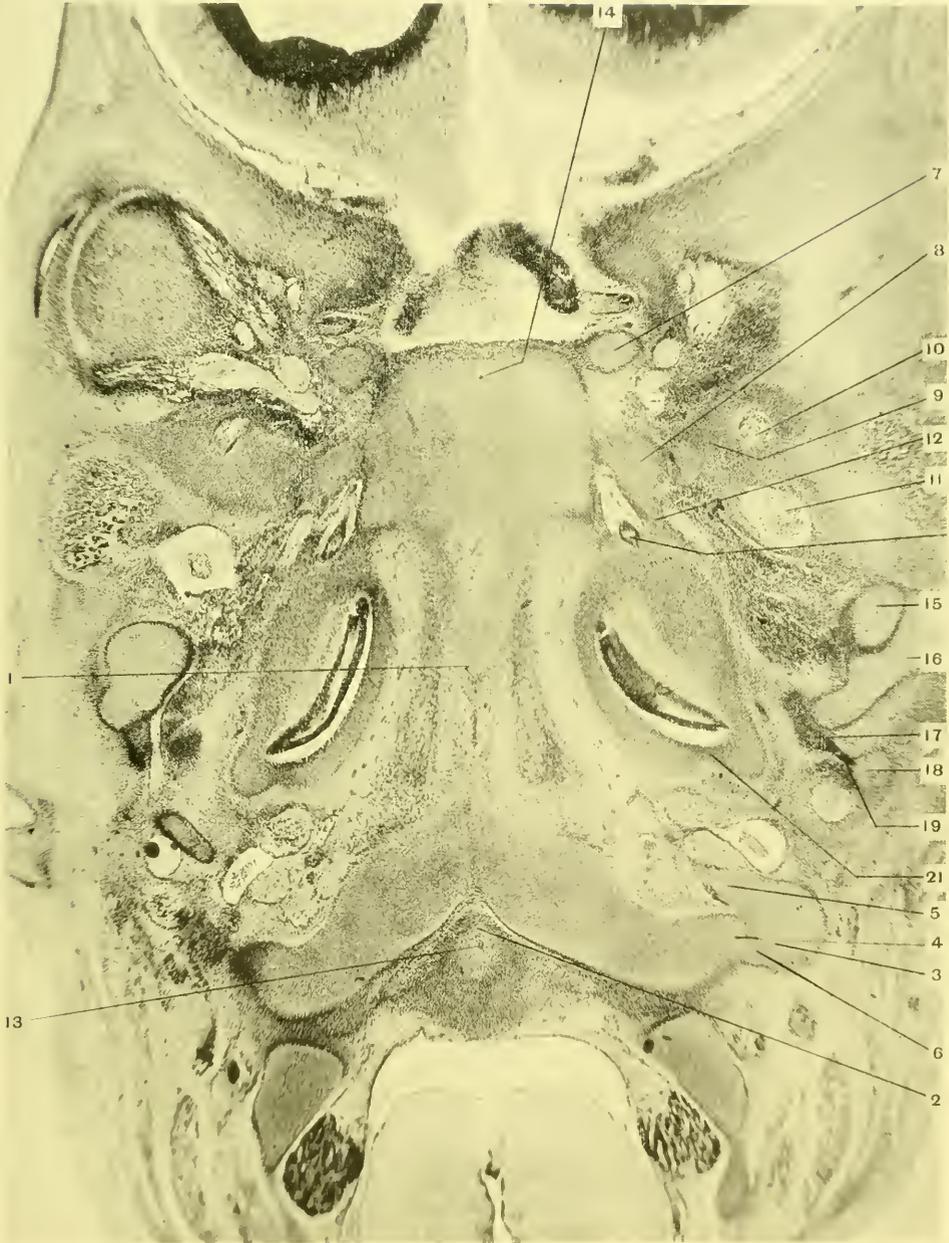
Section of head of human embryo of 20 mm., parallel to planum basale, but at higher level than plate 4.

1, Slender area of basal plate
2, Union of otic capsule to basal plate
3, Anlage of ligamentum dentis epistrophei
4, Costal bar of lamina alaris
5, Transverse process of occipital vertebra
6, Layer of perichondrium separating costal bar from transverse process
7, Cranio-mesial process of paracondyloid process
8, Reichert's cartilage

9, Ala orbitalis
10, Distal extremity, processus ali cochlearis
11, Membranous anlage of side wall of skull
12, Stapes
13, Malleus
14, Incus
15, Interhyale
16, Crista parotica
17, Proximal end, Reichert's cartilage
18, Processus mastoideus
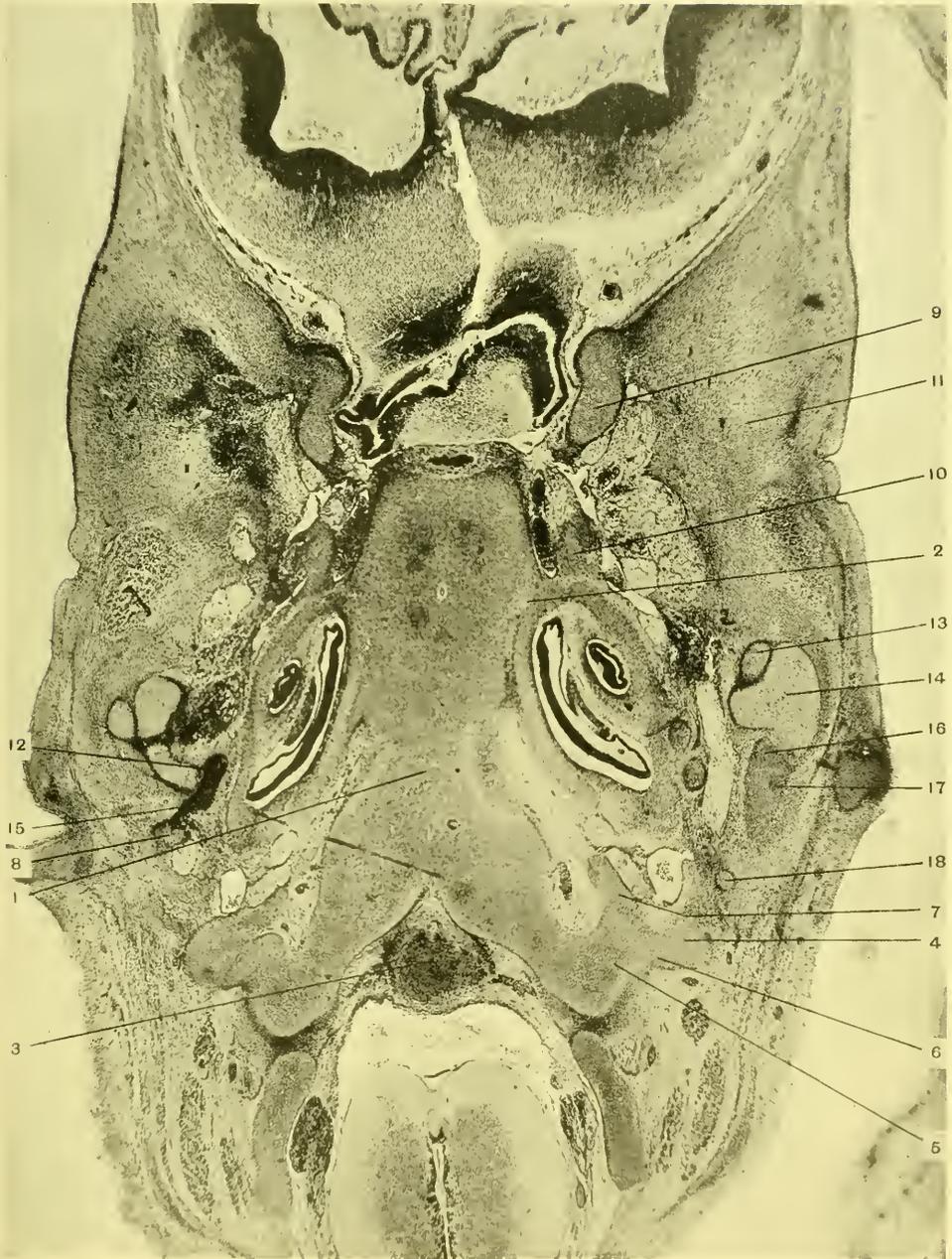
Section of head of 20 mm. human embryo, passing through lower part of eye and through occipital vertebra.

1, Tip of paracondyloid process
2, Lateral masses of occipital verte-
bra
3, Anterior arch of occipital vertebra
4, Chorda dorsalis
5, Dens epistrophei
6, Neural arc h of atlas
7, Ala hypoc hiasmatica

8, Processus alaris of ala temporalis
9, Processus ascendens of ala tempor-
alis
10, Foramen rotundum
11, Nervusopticus
12, Nervusfacialis
13, Chorda tympani
14, Cavum tympani
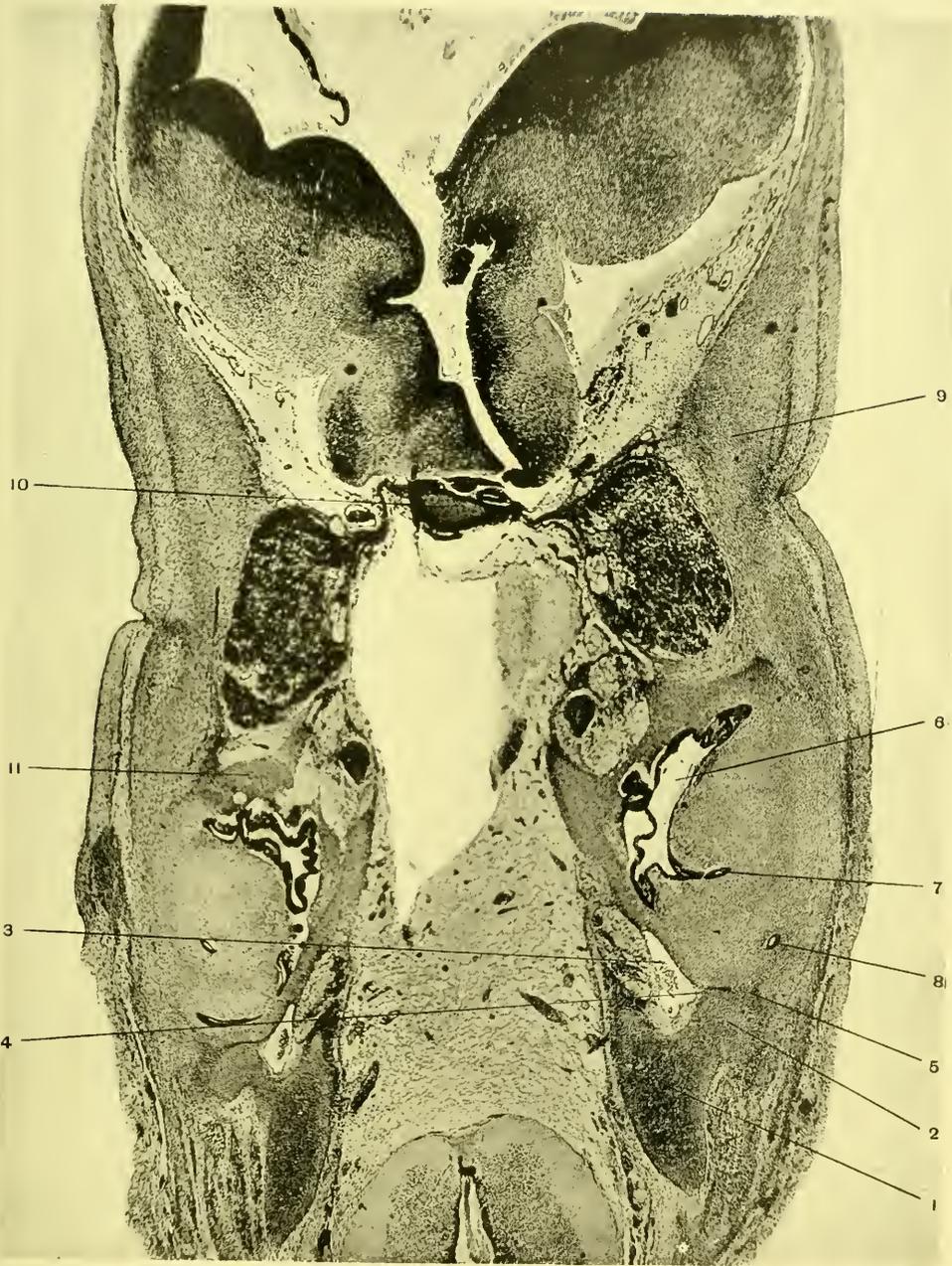
FIGURE 8

Section of head of 20 mm. human embryo passing through epistropheus, atlas, and interorbital septum.

1, Lateral mass of atlas	*6*, Interorbital septum
2, Caudal tips of condyles	*7*, Reichert's cartilage
3, Anterior arch of atlas	*8*, Meckel's cartilage
4, Anterior arch of occipital vertebra	*9*, Manubrium mallei
5, Base of densepistrophei	*10*, Ventral tips of alae temporales

FIGURE 9

A. Section through nasal region and lower jaw of 20 mm. human embryo.

1, Septum nasi
2, Paries nasi
3, Maxilla
4, Reichert's cartilage

5, Mandibula
6, Cavum oris
7, Tongue

B. Chondrification of atlas.   Section through atlas and epistropheus of a 20 mm. human embryo

1, Lateral masses of atlas
2, Anterior arch of atlas

3, Body of epistropheus
4, Arch of epistropheus
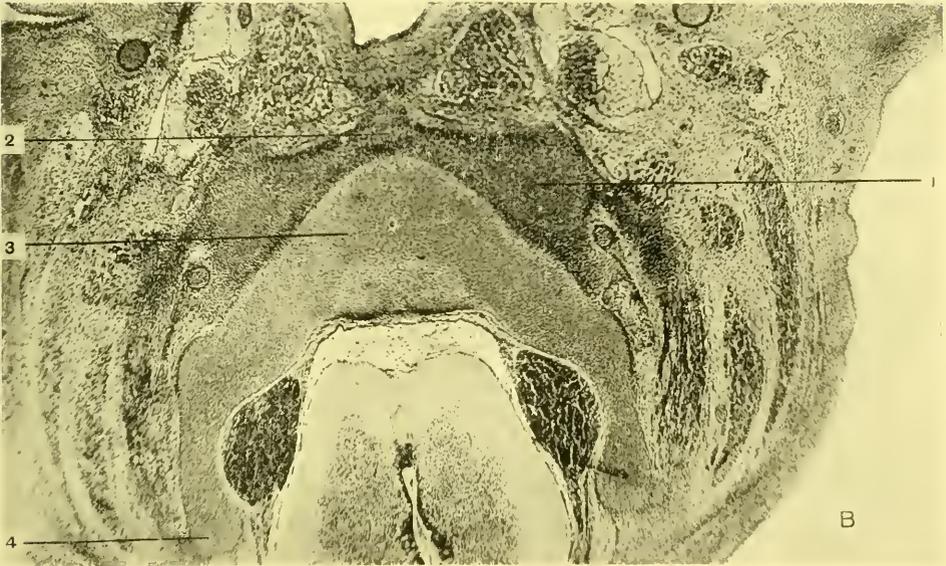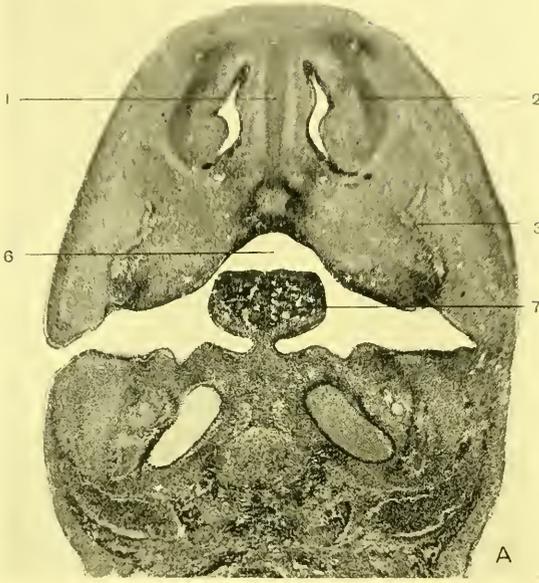
TOOTH DEVELOPMENT IN DASYPUS NOVEMCINCTUS

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FIFTEEN TEXT FIGURES AND FOUR PLATES

A. INTRODUCTION

Much interest has been attached to the development of teeth in the armadillo since 1874 when Tomes first described an enamel organ in the lower jaw of an embryo of Dasypus novemcinctus. The teeth had always been described as possessing no trace of enamel, and therefore the discovery of an enamel organ was received with much surprise. Between the years 1891 and 1895 interest was kindled anew by a series of papers on the subject by Röse, Leche, Ballowitz, and Kükenthal. In 1904, Spurgin, at the University of Texas, also worked upon tooth development in this form. These investigators added much to our knowledge of the subject, and for the most part their observations were correct. But many questions remained unanswered owing to the fact that none of these investigators possessed a series of embryos

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sufficiently complete to enable them to work out the full history
of tooth development, and they were obliged to draw their con-
clusions from a few isolated stages.

Through the kindness of Professor Newman in giving me
access to his large collection of embryos of Dasypus novemcinctus,
I have been able to choose a series of stages which gives a com-
plete history of events from the time of the laying down of the
dental lamina until birth. I have examined the following em-
bryological stages: 30 mm., 35 mm., 48 mm., 50 mm., 53 mm.,
55 mm., 61 mm., 65 mm., 71 mm., 75 mm., 78 mm., 82 mm.,
83 mm., 92 mm., 100 mm., 103 mm., and 108 mm. These
figures give approximately only the relation of these stages to
each other, for two stages showing little difference in size, may
show a great difference in degree of tooth development; and on
the other hand, two stages differing much in size may show little
difference in that respect. That degree of development is not
always associated with size may be seen from the fact that the
100 and the 108 mm. foetuses were full term. My stages repre-
sent a complete series in tooth development, and there are no
wide gaps. Unfortunately the picture after birth is not com-
plete, as I was not able to procure any stages between that of
the foetus at full term, and post embryonic stages of at least
four months after birth. It is impossible to breed these animals
in captivity as the mother devours her young as soon as they are
born, and the young cannot be reared by hand. In the wild, as
soon as the young are born, the mother retires with them to almost
inaccessible places in the rocks, and does not bring them out
until they are several months old. Mr. Johns, of the Armadillo
Curio Company in Boerne, Texas, tried in vain to get some of
these young animals for me. Through this same collector, I
have succeeded in obtaining a large number of young animals
several months old (probably 4 to 6). However, these skulls
have proved of little value except for gross observations, being
very unsatisfactory for histological study. During the journey
from Texas, the containers had broken, and most of the formalin
in which the animals were fixed had leaked away. Moreover
it was almost impossible to decalcify the jaws sufficiently for

sectioning, and by the time decalcification was complete, they were of little use for histological purposes. I was able, however, to obtain a few good sections which have proved of great value.

This gap in the series between birth and late post-embryonic stages is most regretable but has not seriously interfered with the understanding of the tooth ontogeny. As will be seen, the condition at birth and the stages leading up to this condition give a clear prophecy of coming events so that with the facts which may be gleaned from the post-embryonic stages one may be sure that he has a very accurate picture of what has happened between the two stages.

B. DESCRIPTION OF TEETH IN POST-EMBRYONIC SKULLS

Before proceeding to the review of the literature or to the results of my own investigations, it may be well to describe briefly the facts concerning the dentition of Dasypus novemcinctus as they may be ascertained from a survey of the dried skulls (text fig. 1). The teeth occur in the posterior part of the skulls and the dental formula is 7/7 or 8/8, depending on whether or not the last back tooth has been erupted, as this tooth erupts some time after the others. As this last tooth erupts in the lower jaw before it does in the upper, at certain stages the dental formula may be 7/8. The teeth are so arranged in the jaws that the first front tooth of the lower jaw has no corresponding tooth in the upper jaw with which to articulate (see text fig. 1); the second front tooth of the lower jaw articulates with the first front tooth of the upper jaw. The teeth alternate with each other as shown in text figure 1, so that the last back tooth of the lower jaw articulates with the next to the last back tooth of the upper.

Although it is maintained that in Dasypus peba the first upper front tooth is situated in the premaxilla, I can state with certainty that both in the embryonic and post-natal stages of D. novemcinctus the first upper front tooth is situated in the maxilla, and some distance back from the premaxilla so that a considerable diastema is left between the two. This appears plainly in text figure 1.

In teeth worn with use, it is impossible to determine the number of cusps, but in a young specimen four or five months old the first, second and eighth teeth of the lower jaw are one-cusped, and the others are two-cusped, with a higher lingual and lower labial cusp. In the upper jaw the first and eighth teeth are one-cusped, the others being two-cusped. In each case the eighth tooth is smaller and narrower than the others.
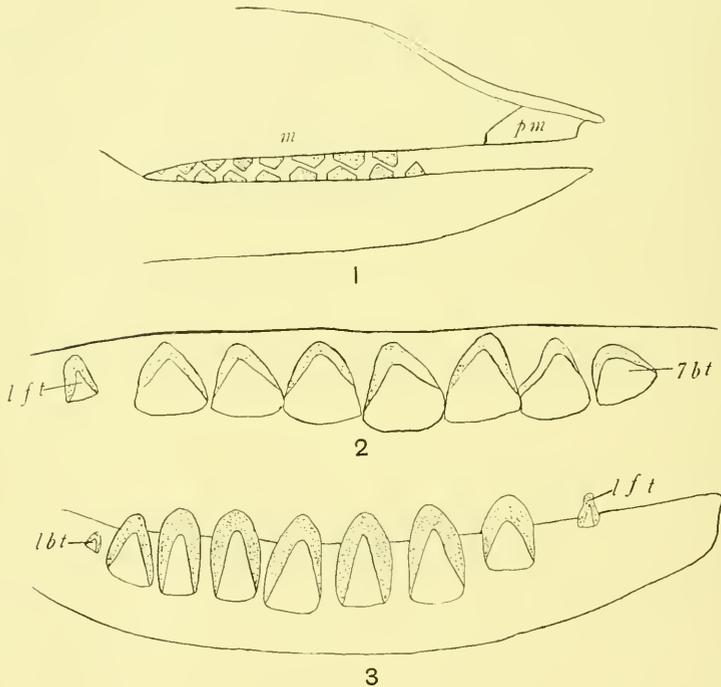
Fig. 1 Diagram showing teeth in skull a few months after birth. *m*, maxilla; *pm*, premaxilla.

Fig. 2 Camera lucida drawing of germs of sixth front tooth and the seven back teeth in the jaw of an 82 mm. embryo. *lft*, last (6th) front tooth; *7bt*, seventh back tooth. × 15 (reduced ½).

Fig. 3 Camera lucida drawing of lower jaw a few months after birth showing the eight back teeth and the last front tooth which is usually shed at a younger stage. The eighth back tooth (*lbt*) had not erupted. Notice the superficial position of the last front tooth. *lbt*, last (8th) back tooth; *lft*, last front tooth. × 5 (reduced ½).

C. NUMBER OF TOOTH GERMS PRESENT IN THE EMBRYO

1. Number of tooth germs in the embryonic lower law

The literature on dentition in the armadillo reveals the fact that previous investigators have given varying accounts of the number of tooth germs present in the embryo. They seem fairly unanimous in agreeing that the germs of 'eight back teeth' are present, but the number of 'rudimentary incisors' reported varies from none at all to seven (Leche). There also exists much confusion regarding the classification of the armadillos and in many cases it is impossible to discover whether investigators are describing the same or different species. Many of the conflicting results are doubtless due therefore to the fact that different species were examined.

My work was done entirely upon D. novemcinctus and in comparison with those of previous investigators my results are strikingly uniform. As soon as tooth buds are definitely formed, there are found to be thirteen as a maximum and twelve as a minimum number. This number, however, does not include the last back tooth, which up to near the time of birth is represented only by a backward continuation of the dental lamina beyond the last definite tooth bud. At birth this backward continuation of the dental lamina shows a definite enlargement which corresponds to the future tooth germ. In all these early stages, whether twelve or thirteen tooth germs occur, there are present in front of the first tooth germ, scattered groups of epithelial cells which plainly represent a degenerating dental lamina which extended into this region before the formation of definite tooth germs. This would indicate that the ancestors of Dasypus possessed teeth in this region, and that, as observed by Leche, a reduction of teeth is now going on. That this reduction is now in progress in also evidenced by the fact that when twelve tooth germs instead of thirteen are present, it can be shown that it is the most anterior tooth germ which has failed to develop in the former case. It seems to be a matter of chance, whether or not this front tooth develops. I found it in approximately 50 per cent of the embryos which I examined. The chances are about

equal as to whether the dental lamina in front of the second tooth will give rise to a tooth germ or degenerate. It is also conceivable that this dental lamina might sometimes give rise to teeth in front of the first tooth so that very likely Leche did observe fifteen tooth germs in a 46 mm. embryo; probably the first two would not have developed far. Leche's Tatu peba may also represent a different species from D. novemcinctus.

As for homologizing these teeth with those of the adult animal, there are present in all embryos in which teeth have reached any degree of development, seven large teeth in the posterior part of the jaw. They are distinguished from the other tooth germs by their larger size, the above-mentioned posterior continuation of the dental lamina beyond the last one of them, and the fact that there exists a marked diastema between the first of these seven teeth and the next tooth anterior to it. From their position in the jaw, their shape and form, it is evident that these seven teeth represent the seven functional teeth which are present in the young skull, and posterior to the last of which the last functional tooth is erupted five or six months after birth (compare text figs. 2, 3, and 4). In front of the diastema, anterior to the first of these functional back teeth, there occurs in the embyro a tooth which during development becomes much larger than the other front teeth, although it never attains the size of any of the functional back teeth (text figs. 2, 3, and 4). In every respect excepting size, this tooth outstrips all the other teeth in its development. Thus it is the first tooth to acquire enamel and dentine. Later in this paper, I have given a full description of this tooth and will describe it here only as far as is necessary in order to show the errors it has caused in previous interpretations of tooth homologies in Dasypus. See text figures 2, 3, 4 and 10, which show the relative sizes of the different tooth germs at various ages. At birth this tooth shows no evidence of decrease in its developmental activity and, a priori, there is no reason for supposing that it will not become one of the functional back teeth; in fact at birth it shows every evidence of being on the verge of eruption, and moreover up to a later period of embyronic development this tooth germ is not noticeably smaller than those

which do develop into functional back teeth. For these reasons it is not strange that all previous investigators have considered this to be one of the functional back teeth. Thus Röse describes eight back teeth and two rudimentary incisors in a 6 cm. and 7 cm. embryo. A comparison between Röse's figure 10 and my figure will show the striking resemblance between Röse's 'first back tooth,' and the last front tooth as I have described it. Kükenthal describes eleven back teeth of which three are rudimentary. Leche also mentions eight well-formed tooth anlagen which he considers to represent functional back teeth; and Spurgin claims to have found thirteen teeth in each half jaw, of which eight are back teeth and five rudimentary incisors. Spurgin also says that the eighth back tooth, which has no predecessor in the milk dentition, has a well-developed enamel organ. As I shall show later the eighth back tooth is indeed not represented in the milk dentition, but, since Spurgin mistook the last front tooth for the first functional back tooth, his 'eighth back tooth' is in reality the seventh. That his 'eighth back tooth' could not possibly have been the true eighth is proven by the fact that during the entire embryonic life I have found the eighth back tooth to be represented only by a backward continuation of the dental lamina, which does not begin to take on the appearance of a tooth germ until about the time of birth (100 to 108 mm.) which is much later than Spurgin's oldest embryo (90 mm.). Spurgin evidently overlooked this backward continuation of the dental lamina entirely.

My reason for not considering this tooth to be a functional back tooth is that it is not ordinarily found in the jaw after tooth eruption; at least in the jaws of animals four or five months old, and the functional teeth of these animals can, as I have said before, be homologized with the seven large teeth in the posterior part of the embryonic jaw. The ultimate fate of the last front tooth can be ascertained only by a study of stages of development between the time of birth and the youngest post-embryonic stages that I have been able to procure. I have actually found this tooth in two of the twenty-nine post-embryonic skulls in my possession. In one case the tooth was not erupted, but its
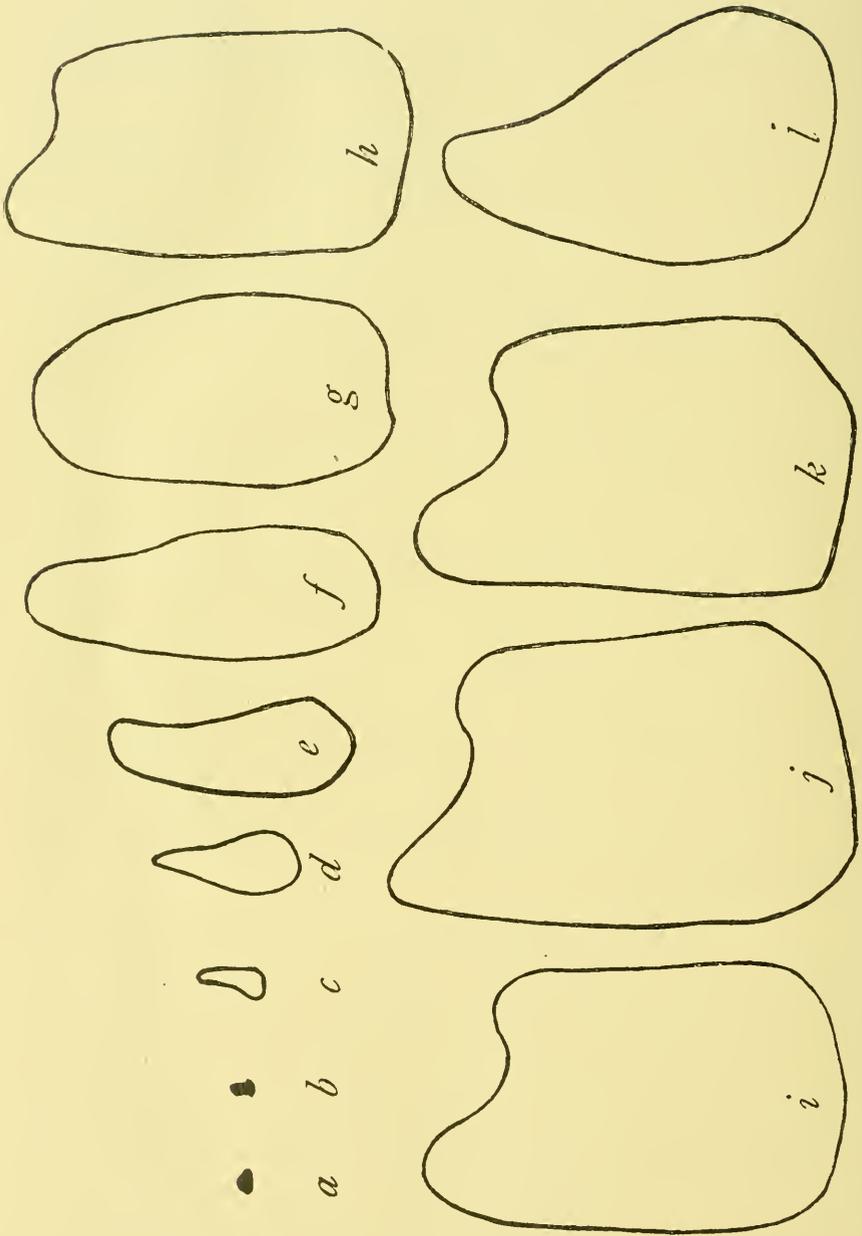
Fig. 4 Diagrammatic sections showing relative sizes of teeth at birth. (108 mm.). *a–e,* 2nd, 3rd, 4th, 5th, and 6th front teeth (the 1st front tooth was not developed in this animal); *f–l,* back teeth 1–7. 31.

apex could be felt just under the surface of the gum. In the other case the tooth was erupted (lower jaw of this animal in text fig. 3.) A glance at text figures 2 and 3 will show that this tooth is much more superficially placed in the jaw than are any other of the back teeth. This tooth is evidently either resorbed or else erupted and shed very soon after birth. Reinhardt says that the last front tooth is sometimes retained in the half grown animal.

The other front teeth become successively smaller toward the anterior end of the jaw. The first two are always very small, and in fact, the first three actually decrease in size between a 78 mm. stage and a 108 mm. stage. Since these teeth are so small and inconspicuous it is easy to overlook them with an unsatisfactory stain unless very careful observations are made. This probably accounts for the fact that previous investigators have usually failed to describe more than three or four of the small front teeth. It is, however, difficult to understand why in a 78 mm. embryo, Leche failed to find any of them with the exception of the last one which he considers to be the first back tooth. It is, of course, quite possible that in an animal in which there is occurring a progressive reduction of these front teeth, fewer teeth than usual may sometimes be formed, so that these investigators may have observed all of the tooth germs that were present in their material. However, this explanation would scarcely account for an entire absence of the first five front teeth in a 78 mm. embryo such as Leche describes.

2. Number of tooth germs in the embryonic upper jaw

So far as I am aware no account has been given of the number of tooth germs present in the embryonic upper jaw with the exception of Spurgin's statement that he cound find no trace of rudimentary incisors in either an 8.5 or 9 cm. embryo. From my own investigations, I can say that there are present in the embryonic upper jaw seven well-formed tooth buds which can be homologized with the seven back teeth of the post-embryonic upper jaw a few months after birth. There is also present the

backward continuation of the dental lamina which gives rise to an eighth back tooth. As I shall show later in this paper there also occur in the premaxilla from three to five epithelial cysts which probably represent front teeth in the last stages of degeneration.

D. DEVELOPMENT OF THE FUNCTIONAL BACK TEETH

1. History and functions of the enamel organ

A review of the literature on the development and fate of the enamel organ makes it evident that the following problems remain to be solved:

1. Does the enamel organ form any secretion or is its only function that of giving form to the developing dental papilla?

2. If a secretion be formed, what is its nature? Is it a thin structureless membrane, as Röse would have us believe, or is there a formation of true enamel, as Spurgin claims?

3. If the enamel organ forms any kind of a secretion, what is the time relation between the formation of this secretion and the disappearance of the various parts of the enamel organ?

The history of the enamel in the upper jaw is exactly similar to that in the lower, except that, as Spurgin has already pointed out, the development in the lower jaw is usually ahead of that in the upper. For this reason, I have confined the following account to the events in the lower jaw. For the sake of uniformity, I have, with one or two exceptions, used the sixth back tooth as the basis for my figures. This is an arbitrary choice, and any one of the functional back teeth in the upper or lower jaw could just as well have been used. For figures showing the different stages in the development of the functional back teeth I refer the reader to figures 1, 2, 3, 4, 5, 6, 7 and 8, and text figure 4.

My investigations show that the origin of the enamel organ in D. novemcinctus is similar to that in other mammals—all parts developing (fig. 3) although there is an early disappearance of the enamel pulp. These results are in agreement with those of Röse, Ballowitz, and Leche. Figure 3 shows all parts of the enamel organ present in a 78 mm. embryo, while figure 4 shows

that the enamel pulp has disappeared in the 82 mm. embryo, leaving a compact layer of cells representing the outer and inner enamel epithelium and the stratum intermedium. Thus at first the enamel organ is composed of an outer enamel epithelium stellate reticulum, stratum intermedium, and inner enamel epithelium. The ameloblasts are always unusually short, but, as has been pointed out by Tomes, there is a great variation in the length of ameloblasts; they are longest "when enamel formation is most active and a material thickness of enamel is to be formed" (Tomes '14, p. 169). In D. novem. as shown in figures 4, 5 and 7, an exceedingly thin layer of enamel is always deposited. The enamel organ disappears early, but this is due to the fact that its function is performed early, and so there is no necessity for its longer persistence. I have never been able to demonstrate the presence of stellate reticulum over any part of a tooth possessing a visible deposit of enamel. The stellate reticulum, then, is the first part of the enamel organ to disappear, the outer and inner epithelia finally coming into contact, separated only by the stratum intermedium, as I have described in connection with the 82 and 83 mm. embryos (fig. 5). To quote from Tomes,

The destination and function of the stellate reticulum is not very clear. Enamel can very well be formed without it, as is seen amongst reptiles and fish, and even in the mammalia it disappears prior to the completion of the tissue so that a great deal of enamel is formed after the internal and external epithelia have come into contact.

The enamel which is deposited in the teeth of the armadillo then evidently corresponds to the last formed enamel of other animals, which may be deposited after the disappearance of the stellate reticulum.

I may state here that I have never observed the presence of blood vessels within the stellate reticulum; the rich supply of blood vessels in the vicinity of the enamel organ of the older foetuses is derived from the surrounding connective tissue which is in direct contact with the cells of the enamel organ.

As to the ultimate fate of the outer enamel epithelium and stratum intermedium, I can only say that prior to the formation of enamel, the outer enamel epithelum comes in contact with the

stratum intermedium and inner enamel epithelium, as shown in figure 5. During the period when the enamel is forming there is present over the ameloblasts a compact layer of cells which probably consists of outer enamel epithelium and stratum intermedium, the stellate reticulum having entirely disappeared, as stated above. The reason that, with the exception of Spurgin, previous investigators found no enamel is obvious. Tomes found none because none had been deposited. He lays great stress on the fact that none would have been deposited because the inner and outer epithelia are in contact, and that there is no trace of a stellate reticulum. But as I have said before, this condition of the enamel organ is reached before any trace of enamel can be seen. Röse said that enamel is not deposited, but that in its stead there is secreted a thin structureless membrane, which corresponds to Nasymth's membrane. The enamel before calcification (fig. 4, *LE*) does have the appearance of a thin structureless membrane, and Röse must have seen the first formed layer of enamel before calcification had taken place. That the enamel is by no means a structureless membrane can be readily seen by a glance at figure 4, *FE*.

The reason that Ballowitz found no enamel is that he had a stage which was far too young for enamel deposition to have occurred. In his younger specimens, the four layers of the enamel organ were still intact. In his older stages, Ballowitz describes the disappearance of the outer enamel epithelium and stellate reticulum, and concludes that, since these have disappeared, enamel deposition would never have occurred, in spite of the fact that Tomes' processes were present. Had he examined a slightly older stage, he undoubtedly would have found enamel.

Spurgin is apparently the only one who has previously described enamel in connection with these teeth. He states that the first deposition of enamel takes place before the disappearance of the stellate reticulum. As proof of this, he has shown a figure (fig. 3), which represents a cross section through the first back tooth of the lower jaw. A slight deposition of enamel has taken place, and the enamel organ is composed of a compact layer of cells

which exactly resembles the compact enamel organ that I have
shown in figure 5.   In my specimens there is certainly no stellate
reticulum represented in this mass of cells, but it is composed of
outer enamel epithelium, stratum intermedium, and inner enamel
epithelium.   I have applied the term enamel to the secretion
deposited by the ameloblasts, for, as far as I have been able to
prove, this secretion is true enamel.   Spurgin also says that true
enamel is deposited upon these teeth, although I do not know
what criteria he used to determine that it is enamel.   The secre-
tion which I have termed enamel is certainly deposited through
the agency of Tomes' processes, and its appearance is exactly
like that of the enamel on the developing tooth of the cat.   I
have never been able to demonstrate satisfactorily the presence
of enamel prisms, but this is probably due to the fact that cal-
cification is not yet complete and also to the action of the decal-
cifying agent.   However, in favorable specimens, as shown in
figure 4, the enamel is sometimes seen to be composed of darker
areas which have separated from each other, and between which
lighter areas appear.   I have interpreted these darker areas as
representing the enamel prisms.   Any breaks occurring in the
enamel are always in the direction that would be taken by enamel
prisms.   Decalcification destroys all prismatic structure, so that
examination under a polarizing microscope is useless.   Attempts
to grind down the calcified teeth have so far been unsuccessful
because of the extreme thinness of the enamel coat, which causes
it to break off in the process of grinding.   The difficulty of deter-
mining the presence of enamel has already been recognized by
Tomes, who says (p. 30), "Although it might appear as an ex-
ceedingly simple matter to determine whether or not a tooth is
coated with enamel, as a matter of fact in practice it is not always
easy to be certain upon this point."   Therefore, since I have no
evidence against the conclusion that the substance is enamel,
and since it is secreted through the agency of Tomes' processes,
and looks like the newly formed enamel in the cat's tooth, I feel
that I am justified in applying the term enamel to the secretion
which covers the dentine in the teeth of the armadillo.

Besides the separated darker areas described above, there are always visible in the enamel a large number of fine, closely set lines which run parallel to the surface of the tooth. These lines evidently represent different strata of enamel deposition (fig. 4, *FE*).

Summary. The history and fate of the enamel organ in the teeth of D. novemcinctus is similar to that of other mammals. A thin layer of enamel is deposited, and correlated with this fact, we find unusually short ameloblasts, and an early disappearance of the stellate reticulum.

2. History of the tooth cusps

Previous investigators of the tooth development of D. novemcinctus have said little concerning the history of the tooth cusps, although they have all mentioned the fact that where two cusps are present, the lingual cusp is always higher than the labial. Röse states that in D. hybridus, each of the first two of the seven back teeth are one-cusped, and the other back teeth are bicuspid; and in D. novemcintus all but the two anterior back teeth are two-cusped. Leche states that, since in T. peba the first two back teeth are one-cusped, the milk set must originally have been heterodont. I have already shown that both Röse and Leche mistook the last front tooth for the first functional back tooth, and therefore their statement is that of the functional back teeth, the first alone is one-cusped.

a. History of the cusps in the lower jaw. In a few sections through the first back tooth of my 55 mm. embryo, the dental papilla appears to be slightly two-cusped. If two cusps are present, the lateral one is higher, and this in addition to the fact that, elsewhere in my series, there is no indication of a bicuspid condition in this tooth makes it doubtful whether or not this appearance is an artefact caused by unequal shrinkage in the dental papilla. In all the other stages, as shown in text figure 4 which gives diagrammatic outlines of the teeth at birth, the first back tooth is plainly one-cusped. This tooth also passes through changes in shape similar to those described later for the most

posterior of the front teeth. During its first stages of development, the entrance to the pulp cavity does not increase in size at the same rate as do the other parts of the tooth. The sides of the tooth bulge out because of their increase in tissue, and the tooth also increases in height. As a result, the stages between 70 and 83 mm. give the impression of a tooth with a round dental papilla, and a narrow entrance to the pulp cavity. In later stages, the tooth takes on its definitive shape (text fig. 4, *f*) in which there is a wide entrance to the pulp cavity. This change in shape may mean that the ancestors of the armadillos possessed teeth with a narrow opening to the pulp cavity.

The second back tooth is at first plainly two-cusped (text fig. 5, *1*). In the 78 mm. embryo there is but the slightest trace of the original bicuspid condition. The tooth has apparently lost its two cusps by the upward growth of the groove between them (text fig. 5, *3*). In the 82 mm. embryo (text fig. 5, *4*), the second back tooth possesses but one cusp, the original grove between the cusps having grown upwards until it was level with the cusps. In the 83 mm. embryo the groove between the cusps is plainly visible (text fig. 5, *2*), but as I have said before, the size of this embryo is ahead of its degree of development in other respects. With this one exception, the second back tooth is always one-cusped after the 82 mm. stage, possessing one large rounded cusp (text figs. 5, *4* and 4, *g*).

The next four back teeth (3 to 7) are always plainly two-cusped, with a higher lingual and lower labial cusp (text fig. 4), and so is the seventh back tooth with a single exception; in this case the lingual cusp in both sides of the lower jaw of a 108 mm. embryo has apparently so out-stripped the labial cusp in its growth that the labial cusp is indicated only by a bulge on the side of the tooth, and the tooth is practically one-cusped. I have looked in vain for a similar condition in other embryos. In the unworn teeth of an animal a few months after birth, there seems to be a slight diminution of the labial cusp towards the posterior end of the jaw, and in a few cases, the labial cusp of the seventh back tooth is extremely low, although it is always unmistakably present. It is probable, then, that this extreme diminution of the

labial cusp in the 108 mm. embryo is an exception to the usual condition. It is evidently an exaggeration of the tendency towards reduction of the labial cusp, which is shown in the young animals after birth.
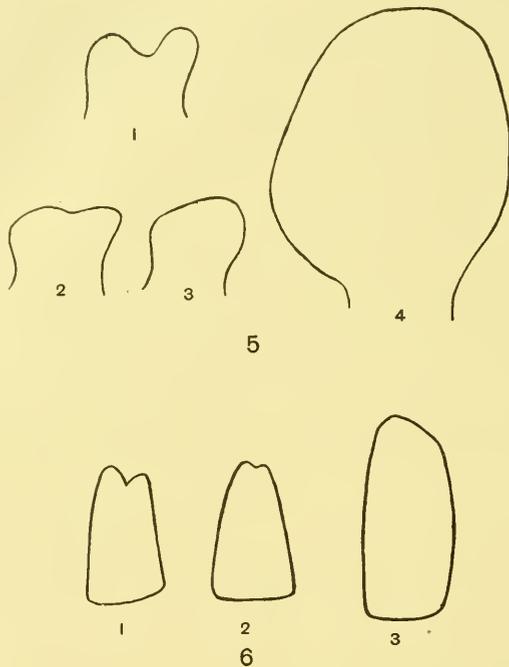
Fig. 5 Diagrammatic sections through second back tooth showing change from a bicuspid to a one-cusped condition. *1*, section, through tooth in 73 mm. embryo; *2*, section through tooth in 83 mm. embryo; *3*, 78 mm. embryo; *4*, 82 mm. embryo. × 88 (reduced ½).

Fig. 6 Diagrammatic sections through eighth back tooth showing change from a bicuspid condition. × 15 (reduced ½).

Text figure 6 shows the conditions in the eighth back tooth, of which I have no history from the time when it is represented by a thickening of the dental lamina at birth (fig. 8) until it is a well-developed tooth in the process of eruption. After eruption, it is always one-cusped, but unerupted teeth show evidence of two cusps, a higher lingual and a lower labial. The younger the

tooth, the more separated are these two cusps. It is therefore apparent that this tooth passes from a two- to a one-cusped condition.

b. *History of cusps in the upper jaw.* In the upper jaw the first back tooth is always one-cusped. Judging from the appearance of the unworn teeth of an animal after birth, the second back tooth eventually becomes single-cusped by the obliterations of the groove between the two cusps. In the 108 mm. embryo, at a time when the second back tooth in the lower jaw has for some time assumed a one-cusped condition, the second back tooth of the upper jaw is still plainly two-cusped, although there is not as marked a distinction between these cusps as in the earlier embryos, showing that the groove between the two is becoming obliterated. The history of the cusps in the other back teeth is similar to that in the lower jaw.

3. Secondary tooth buds

Prior to Tomes, Rapp, Gervais and Flower had called attention to the fact that among the armadillos, Tatu peba is not monophyodont. Hensel, in 1872, from an examination of thirty-five skulls of D. novemcinctus, found evidences of a transition from milk teeth to a permanent set, a change which he says does not occur until the animal has nearly reached the adult stage. Tomes, Kükenthal, Röse, Ballowitz, Leche, and Spurgin, working in the embryonic development of teeth in the armadillos, have described structures arising from the lingual side of the outer enamel epithelium which they interpreted as representing the buds of permanent teeth—but the further history of these structures has never been worked out.

My own investigations have revealed the fact that secondary tooth buds arise normally in both upper and lower jaws from the lingual side of the outer enamel epithelium. In the lower jaw they are first distinguishable in a 55 mm. embryo, and in the upper jaw they can be seen in a 61 mm. embryo. They appear first in connection with the most anterior teeth, and last in the seventh back tooth. During embryonic development, these tooth buds never progress beyond the stage in which the cross

section presents the appearance of a narrow column of cells (figs. 7 and 9) possessing an outer layer of columnar cells and an inner layer of long narrow cells with their long axes at right angles to those of the cells of the outer layer. This lack of activity on the part of these tooth buds during embryonic life is associated with the well known fact that the permanent teeth are not erupted until relatively late in the life of the animal.

The gap in my series between birth and a stage several months after birth leaves us ignorant as to the intermediate stages in the development of these permanent teeth, but sections through the permanent teeth of the animals a few months after birth (fig. 10) show that their development must have been similar to that of their milk predecessors. They are rootless, of persistent growth and possess normal tubular dentine. These permanent teeth also possess a thin layer of enamel. Enamel deposition is still in progress, so that I do not know how much is formed.

In the young animals the permanent teeth are found lying in grooves on the lingual side of the first seven back teeth. These grooves have been formed by the absorptive action of the permanent teeth. Text figure 7 shows the relation of the permanent and milk teeth. Tomes shows a picture of a still later stage in which the permanent tooth has absorbed the entire center of the base of the milk tooth. In the Field Museum of Natural History, I have examined a large number of skulls in which the permanent teeth were erupted. In some of these teeth, I found, at both anterior and posterior ends, a thin scalelike remnant of the milk tooth. This proves that the permanent tooth grows up through the center of its milk predecessor. The most anterior of the permanent teeth is one-cusped; the others are bicuspid with a higher lingual and lower labial cusp (text fig. 8).

E. DEVELOPMENT OF THE NON-FUNCTIONAL FRONT TEETH OF THE LOWER JAW

From a review of the literature it can be seen that almost everyone who has studied tooth development in the armadillo has described a varying number of poorly developed tooth germs in the anterior part of the lower jaw. Spurgin is the only inves-

tigator who has described the deposition of enamel in connection
with them, and he also appears to be the only one who believes
that they will be erupted. Previously in this paper, I have
described briefly these front teeth and have shown that all earlier
investigators mistook the last of these functionless teeth for one

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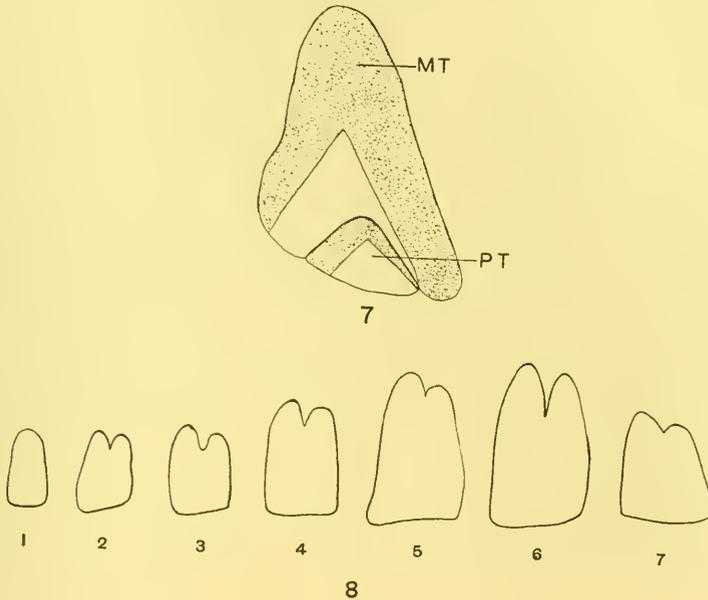
Fig. 7 Diagram of a lingual aspect of milk tooth a few months after birth
showing the position of the permanent successor which lies in a groove at the
base of the milk tooth. *MT*, milk tooth; *PT*, permanent tooth. × 15 (reduced
½).

Fig. 8 Diagram showing the seven permanent teeth a few months after birth.
These teeth were lying in grooves on the lingual side of their milk predecessors
and would not have been erupted for some time. *1–7*, permanent teeth 1–7.
× 15 (reduced ½).

of the permanent back teeth. I shall now proceed to a more
detailed account of the development of each of the functionless
front teeth that I have found in my embryos.

Stages in the development of the sixth and last front tooth
may be seen by consulting figures 11, 12, 13 and 14, and text
figures 2, 3, 4, and 10 *a*. The sixth front tooth is always one-

cusped, and, during development, undergoes several conspic-
uous changes in shape (figs. 13 and 14). Some of the minor
alterations in shape are doubtless due to the obliquity with which
the sections were cut, but the change from a low, rather square
papilla with a wide pulp cavity to a round papilla with a narrow
entrance to the pulp cavity is unmistakable. The subsequent
widening out of the entrance to the pulp cavity up to a stage like
that shown in figure 14, and in text figure 10 is equally unmis-
takable. Its development is similar to that of the first back
tooth, except that the parts of the enamel organ are never so
well differentiated, the enamel pulp never forming a stellate
reticulum. Until the 71 mm. stage there is no conspicuous dif-
ference between its size and that of the first back tooth, but after
that stage, the first back tooth takes a rapid lead in size develop-
ment, while the sixth tooth leads in degree of development.
Thus the sixth tooth has received a slight deposition of enamel
in the 75 mm. embryo, while the first back tooth receives no
enamel until after the 78 mm. stage. The formation of odonto-
blasts and dentine also takes place first in the sixth tooth. At
birth, although much smaller than the first back tooth, it has a
relatively larger amount of dentine. As I have stated before,
this tooth may sometimes be found in the jaw several months
after birth (text fig. 3), and it probably erupts and is shed soon
after.

The fifth tooth resembles the sixth tooth in its mode of devel-
opment. From the 71 mm. stage the sixth tooth takes the lead
in size and in each stage becomes increasingly larger than the
fifth tooth, so that at birth it is many times larger (text fig. 4, *e*
and *d*, 10, *a* and *b*). Deposition of enamel in the fifth tooth does
not take place as soon as it does in the sixth, and at birth less
has been deposited, and the enamel organ has likewise totally
disappeared. It goes through changes in shape similar to those
already described for the sixth tooth, although these changes
are here somewhat less marked. At birth it is also long and
narrow. The most interesting point in connection with this
tooth is the behavior of the odontoblasts. In the 71 mm. em-
bryo, when prodentine is beginning to form, some of the odonto-

blasts at the base of the tooth are becoming enclosed within their secretions. In the 78 mm. embryo, some calcified dentine is present, and here again odontoblasts are found imprisoned in the dentine at the base of the tooth. This process continues, so that at birth normal dentine occurs only at the apex of the tooth. In these later stages the pulp cavity is narrow and at birth its entrance is almost closed. The pulp always contains many blood vessels (text fig. 10, *b*).

In the beginning of its development the fourth tooth shows no change from preceding stages. It has the same poorly developed and little differentiated enamel organ. It deposits less enamel than either of the preceding stages. It does not pass through the changes in shape that characterize the fifth and sixth teeth. It always has a rather high narrow papilla; also there is a greater tendency on the part of the dentine-secreting cells to become enclosed within their secretion. There are a few normal dentinal tubules near the apex of the tooth. As development proceeds, the entrance to the pulp cavity becomes narrower from the deposition of cellular dentine at the base of the tooth. At birth the entrance is still open, although it is apparently closed in the 82 mm. embryo. (See text figs. 4 *c*, 10 *c*, and 11 which show this tooth at birth. In 4 it will be seen that there is a much greater difference in size between teeth 5 and 4 than there is in text figure 10.)

In the earlier stages the second and third teeth possess enamel organs similar to those of the front teeth already described. In the 92 and 108 mm. stages an almost imperceptible layer of enamel is found over the cusps of these teeth (text figs. 12 and 13). This layer is so thin that it was some time before I was able to prove to my own satisfaction that any enamel was present. It will be recalled that in an 85 mm. embryo, Spurgin described a much thicker deposit of enamel on these teeth.

In the third tooth, from the 78 mm. embryo on, there is an increasing enclosure of cells within the dentine and an increasing tendency for the entrance of the pulp cavity to be closed by the deposition of dentine. In the 82 mm. embryo, the tooth germ has become an almost solid mass of dentine. In the 92 mm.

embryo, the pulp cavity is not closed, but it is practically closed in the 108 mm. embryo, the inside of the mass being occupied by blood corpuscles and dentine secreting cells (text figs. 12, *10 d*, and *4 b*). This same tendency appears in the first and second teeth but to a still greater degree. (See text figures 13 and 14 which show these teeth at birth. Also see text figure 9 which shows the second tooth in a 78 mm. embryo.)

In the 92 mm. embryo the second tooth may or may not have an entrance to the pulp cavity, but at birth I have always found this tooth represented by a closed mass of dentine, with cells
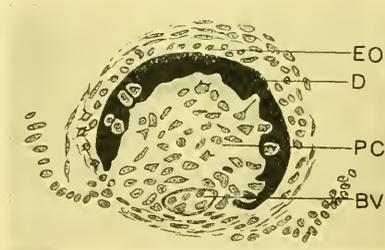
Fig. 9   Section through the second front tooth of a 78 mm. embryo. Notice the enclosure of odontoblasts within their secretion. *EO*, enamel organ; *D*, dentine; *PC*, pulp cavity; *BV*, blood-vessel.   × 560 (reduced ½).

imbedded in it. The center of the mass may not yet be entirely filled with dentine, although dentine deposition is still going on (text fig. 13).

In these later stages it can be seen that the third tooth has a high narrow form while the second tooth is lower and rounded in shape (see text figs. 12, 13 and 14, 10, *d*, *e*, and *b*, also test fig. 4 *a* and *b*). After the 78 mm. stage there seems to be a slight decrease in size in the third and second teeth and at birth they are more deeply imbedded in the substance of the jaw than in earlier stages.

As I have said before, the first tooth may or may not be formed. In the 53 mm. stage an enamel organ for this tooth is evidently forming, and in a 55 mm. stage an enamel organ is present which is as well developed as in the case of the other teeth. In the 61 mm. embryo, I found no trace of a first tooth;

in 71, 73, and 78 mm. embryos, there are present collections of epithelial cells, which from their shape, might be interpreted as degenerating enamel organs. In the 82 mm. embryo, the first tooth is absent in two cases, but in the third, I found it repre-
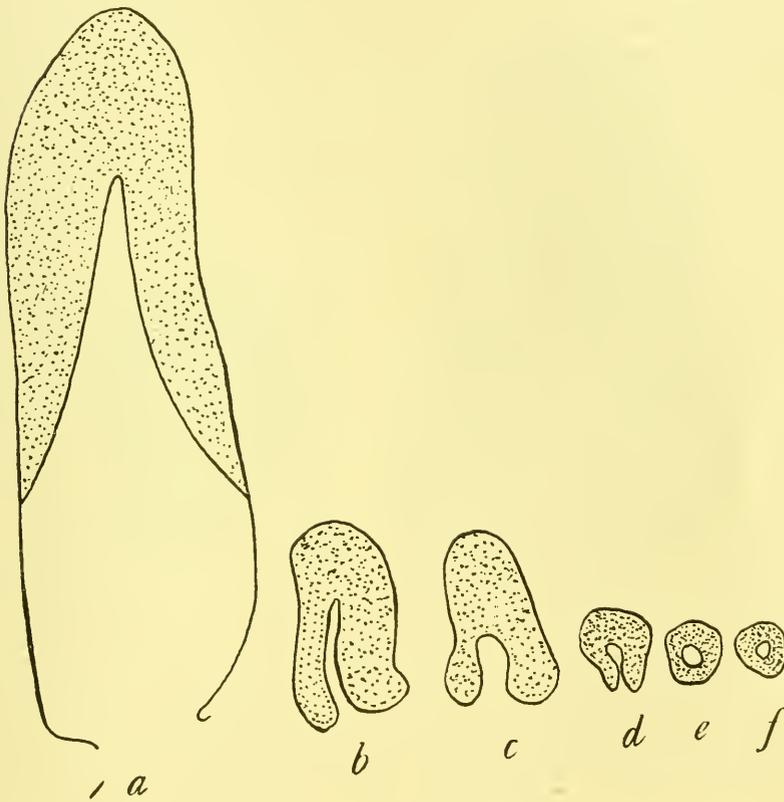
Fig. 10   Diagrammatic section through the six front teeth of a full term fetus. The stippled portion represents dentine.   *a–f*, teeth 6–1 respectively.   88.

sented by a small dental papilla capped with dentine. In the 108 mm. embryo, this tooth again appears—but in one-half of the jaw only; at this stage, it is an almost solid mass of dentine. An exceedingly thin layer of enamel is present and a mass of epithelial cells occurs over the part of the tooth representing the

cusp.   The center of this dentine mass contains blood corpuscles
and odontoblasts which are actively forming dentine, so that at
a slightly later stage one would probably find an entirely solid
mass of dentine (text fig. 14).
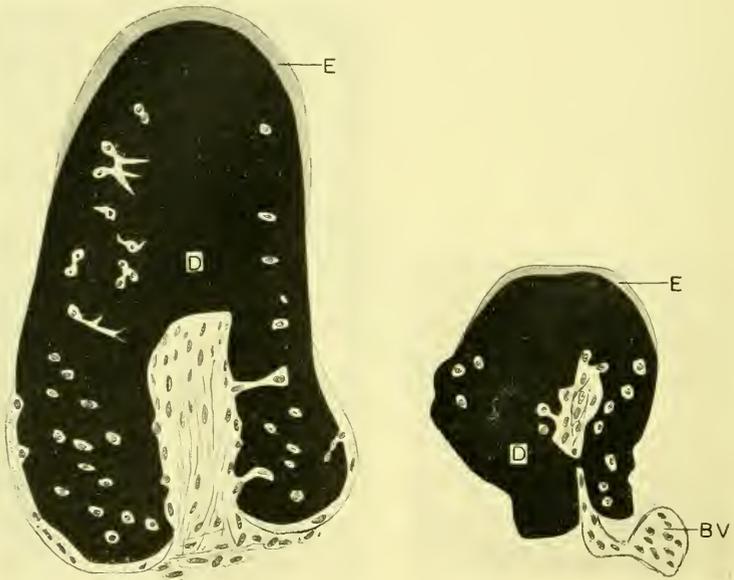
Fig. 11   Section through fourth front tooth at birth showing absence of dentinal tubules and enclosure of odontoblasts within dentine.   E, enamel; D, dentine.   × 560 (reduced ½).

Fig. 12   Section through third front tooth at birth showing cellular dentine.
E, enamel; D, dentine; BV, blood-vessel.   × 560 (reduced ½).

Discussion

From the preceding account it is clear that the anterior part
of the jaw contains either five or six tooth germs.   It is also clear
that toward the anterior end of the jaw dentine becomes less
normal in structure until dentinal tubules are no longer formed,
the cells of the pulp cavity secreting dentine upon all sides of
themselves and becoming inclosed within this secretion.   This
process continues until a mass of dentine is formed, the cells

upon the inside of this mass being able to secrete as actively as
those which are ordinarily destined to become odontoblasts.
The pulp cavity is always rich in blood vessels.

I have searched the literature for analogous cases of odonto-
blasts secreting dentine upon all sides of themselves and becom-
ing inclosed within this secretion.   Cases of this sort are appar-
rently rare, and the only description which seems to apply was
found in Hopewell-Smith's 'Dental Microscopy,' where under
the heading of 'patho-histological dentine,' there is the following
brief description of so-called 'cellular dentine:' "Cells with nu-
clei retained (suddenly caught) in the midst of the deposit."
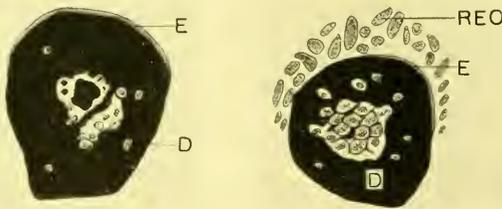
Fig. 13 Section through second front tooth at birth showing cellular dentine
and closure of entrance to pulp cavity. Depositions of dentine can also be seen
within the pulp cavity. E, enamel; D, dentine. × 560 (reduced ½).

Fig. 14 Section through first front tooth at birth. Some of the enamel organ
still remains so that enamel deposition may have still continued. 560 (reduced ½).

The authors also give an illustration of this 'cellular dentine,'
which show an apparently homogeneous substance in which are
imbedded round nucleated cells. The figure does not show any
cell processes, extending into the dentine, such as I have found,
but I believe that this is the same kind of dentine that occurs in
the degenerate front teeth of Dasypus. At least the account
and figure given by the authors more nearly agree with this
dentine than does anything else in the literature. Evidently we
have here an example of very degenerate or generalized odonto-
blasts which, instead of sending out their secreting processes in
one direction only, may extend them in any direction. The
secretion thus formed must be very hard, for in sectioning it is
frequently torn from the surrounding tissue.

In the case of the first three teeth of the lower jaw, it is evident that enamel secretion has almost ceased. The vestigial milk teeth of Perameles and the degenerate milk incisors of the mouse have been described as consisting of a dentine cap upon which no traces of enamel can be found. In Ornithorhynchus, Wilson and Hill find a 'nodule' which has a connective tissue pulp, inclosed by a distinct ring of dentine, outside of which is a layer of columnar cells representing the inner enamel epithelium. The authors consider this nodule to be a vestigial tooth. It probable represents a more extreme case of degeneration than that occurring in these front teeth of the armadillo. In these cases, the authors have made no statement as to whether or not the dentine secreted is normal dentine. Another sign of extreme degeneration of the first three of these functionless front teeth is the fact that while the fourth, fifth, and sixth teeth increase in size during embryonic life, the first, second, and third teeth actually decrease in size so that at birth these tooth germs are smaller than in the 78 mm. embryo. Furthermore, after the 78 mm. stage, the first three teeth recede into the substance of the jaw, this process being more marked from the first tooth backwards. On the contrary, teeth four and five remain about the same distance from the surface, while the sixth tooth advances toward the surface.

The rudimentary front teeth probably belong to the milk dentition. There is no evidence that they ever have predecessors. I have never been able to identify any secondary tooth buds in connection with these front teeth. In their vicinity there are always more or less scattered groups of epithelial cells, which represent the remains of the dental lamina. Sometimes these groups bear a strong resemblance to secondary tooth buds. Röse has described in connection with his two rudimentary incisors lingual outgrowths of the enamel organ which he considers as representing secondary tooth buds. I have found that groups of epithelial cells resembling secondary tooth buds may occur in any relation to the enamel organ of these teeth, and for this reason, I have been led to believe that when such structures occur on the lingual side they represent only a chance grouping

of cells and not a tooth bud. Such a grouping of cells is not con-
stant at any stage of development and, if it ever does represent a
secondary tooth bud, it remains abortive, and does not produce
a permanent tooth bud. These groups of epithelial cells some-
times give rise to cysts which are apparently the exact homologues
of the epithelial cysts which always occur in the premaxilla (figs.
15, 16, 17 and 18).

The homologies of these teeth are doubtful. From its shape,
position in the jaw, and the fact that a marked diastema occurs
between it and the first functional back tooth, the sixth and last
of the front teeth is probably a canine. If shape were the only
criterion, the fifth or even the fourth tooth (text fig. 4, c and d,
10, b and c) might be identified as the canine. But the position
of this tooth is such that it would undoubtedly articulate with
a tooth situated just behind the premaxillo-maxillary suture if
such a tooth were present. Tomes thus defines a canine tooth:
"The nearest approach to a good definition is that which de-
scribes the canine as the next tooth behind the premaxillo-maxil-
lary suture, provided it be not far behind it; and the lower canine
as the tooth which closes in front of the upper canine." The
other front teeth must then represent incisors, and, if we have
correctly homologized the back teeth, the dental formula of the
lower jaw of D. novemcinctus is M 1, Pm 7, C 1, I 6 or 5.

F. DEVELOPMENT OF TOOTH VESTIGES IN THE PREMAXILLA

The literature on tooth development in the anterior part of the
upper jaw seems to be limited to the following quotation from
Spurgin: "Although I carefully examined the sections from the
upper jaw of both embyros (8.5 and 9 cm.), I failed to find any
trace of rudimentary incisors." I have made sections through
the upper jaw of embryos ranging from 35 mm. to 108 mm., and
have a complete history of the events up to birth.

On each side of the jaw of a 35 mm. embryo, there is a con-
tinuous dental lamina which extends for a few sections into the
premaxillary anlage. In the posterior part of the jaw this
dental lamina is connected with the oral epithelium, but loses

this connection about .68 mm. back of the front end of the maxilla. After becoming disconnected from the oral epithelium, the dental lamina is much reduced in size and extends through the substance of the jaw in the form of an irregular oval cord of cells. In a 50 mm. embryo, the posterior end of the premaxilla slightly overlaps the anterior end of the maxilla. Here and there in this region of overlapping, the scattered remnants of
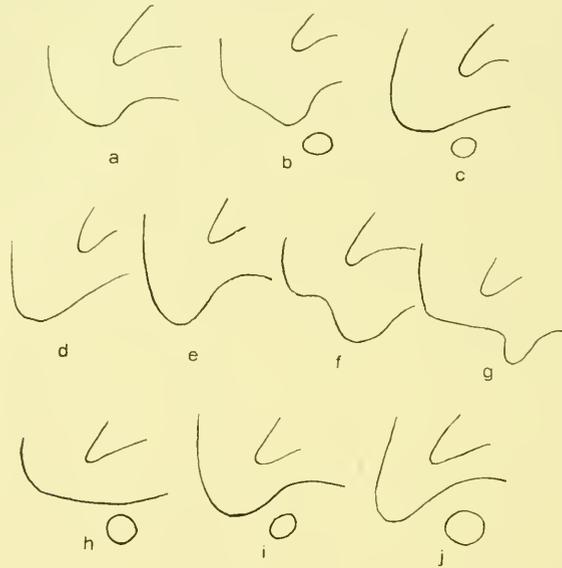
Fig. 15 Diagrammatic sections through posterior part of premaxilla of a 55 mm. embryo showing the formation of the epithelial buds which are destined to give rise to the dermal cysts shown in plate 4, figures 16 to 18.

the dental lamina occur. A similar condition was found in a 60 mm. embryo. But in a 55 mm. embryo which was apparently better developed than the 60 mm. embryo, I discovered two features which are of great interest in the light of subsequent results. Figure 15 shows a series of diagrams through the posterior part of the premaxilla and in that region of the dental ridge in which farther back in the jaw is a slight, upward growth of the oral epithelium. In the next two sections, 15, b, and 15, c, this epithelial bud is no longer connected with the oral epithe-

lium but connection is again established in the fourth section.
In cross section this bud is nearly round; its peripheral cells are
columnar and are like those of the Malpighian layer of the oral
epithelium while the central cells resemble those of the stratum
corneum. Four sections posterior to the one shown in text fig-
ure 15, c is a conspicuous upgrowth of the oral epithelium (text
fig. 15, g) and in the fifth section (15 h) is an entirely detached
epithelial bud which continues through several sections (text
fig. 15, h, i, and j). The structure of these buds is shown in
figures 15 and 16. In this vicinity are a few remains of the den-
tal lamina but these have no connection with the epithelial
buds.

In the same region of the premaxilla of a 65 mm. embryo there
occur four epithelial buds similar to those described above.
Two of these buds are still attached to the oral epithelium.
Figure 15 shows one of these attached buds, and figure 16 shows
a bud after it has become detached. Anterior to the first of these
buds is an upgrowth of the oral epithelium which is evidently
destined to give rise to a fifth bud.

Figure 17 shows the structure of one of these buds or cysts in
an 82 mm. embryo. The cysts are larger than in preceding
stages. The periphery still resembles the Malpighian layer of
the oral epithelium, while the inside has a structure like that of
the stratum corneum The cells of the central portions are
becoming vesicular. The nuclei are irregular and show signs
of degeneration, and the cell walls are thickened as though being
transformed into horny material. This appearance is likewise
presented by the stratum corneum of the oral epithelium.

In older embryos, the outside layer of the oral epithelium is
composed of a clear horny stratum which has arisen through the
cornification of the peripheral cells of the stratum corneum. If,
instead of being allowed to lie in a stright line, a strip of this oral
epithelium had been rolled up until the two ends met, the ap-
pearance would be similar to that of the epithelial cysts found
in these later stages. The peripheral layer takes the same
stain as the Malpighian layer of the oral epithelium. The cells
of this peripheral layer become much flattened, and at birth

have almost entirely degenerated. Immediately within the extreme outside of the cyst, the appearance is like that of the deeper layers of the stratum corneum. Here the cyst is composed of long, flat, concentrically arranged cells containing flattened nuclei (fig. 18). Within this is a clear horny layer in which no nuclei are visible and which looks exactly like the horny layer on the extreme outside of the oral epithelium. This layer is very resistant and splits easily, forming concentric layers. The center of the cyst is less resistant and instead of splitting, it usually becomes much wrinkled as if it had shrunken away from the outer layers. The central portion of the cyst has a granular appearance, instead of being clear and homogeneous. This granular portion is evidently derived from the degenerated central cells which in younger stages were swollen and vesicular.

Discussion

These cysts that I have described evidently belong to the general class of dermoid cysts which are found in various parts of the body. These cysts are supposed to arise from epithelial tissues which during development have become included in the mesoderm. In this new environment these inclusions produce structures which are characteristic of the epithelium on the outside of the body, such as hair in man, bristles in swine, and feathers in birds. The cysts occurring in the gums of D. novemcinctus conform to the descriptions of the so-called 'epithelial pearls,' which are described as epithelial inclusions which remain as simple collections of cells or at least go no farther than the transformation of the cells into horny substance. James shows a photograph of an epithelial pearl which might well be a photograph of one of the cysts in the premaxilla of the armadillo. The following account of the formation of a horny epithelial pearl, taken from James, is an exact description of the formation of the cysts in the armadillo. "The central cells proliferate, the outer ones become flattened and elongated so that together they form long coiled fibers; later the central cells show marked degeneration and become swollen and indistinct." Figure 18 plainly shows a differentiation between the central

granular portion of the cysts and an outer layer which splits into concentrically arranged fibers.

The connection between epithelial pearls and tooth formation has long been recognized. Bland-Sutton believes that the frequently occurring mesopalatine teeth develop from epithelial pearls which have arisen from inclusions of the oral epithelium in the middle line of the hard and soft palate. This same writer regards these epithelial pearls as identical with enamel organs, although they sometimes form horny substance instead of enamel. Bland-Sutton says that in one specimen of ovarian tumor "it was impossible to trace every stage between a typical epithelial pearl and an enamel organ. In a series of sections some showed the ingrowth from the surface of a loculus, in a few sections pearls were visible composed of large epithelial cells, whilst others exhibited laminae of horny material and in some of the sections a developing tooth with its papilla, enamel organ, and gubernaculum could be seen." The author here calls attention to the fact that the pearls apparently arose as an independent down-growth from the surface epithelium, and not from a chance inclusion of the epithelium as usually described.

In connection with the disappearing dental lamina, epithelial pearls have been described by Magetal, Röse, and Leche, and also by Turner and Colyer who believe that these remains of the dental lamina give rise to tumors of the jaws when irritated by pressure or by septic products absorbed from a decaying tooth. In an early part of this paper I have mentioned the fact that Röse and Leche described 'epithelial pearls' in the anterior portion of the lower jaw of the armadillo.

A still more intimate relation between epithelial pearls and teeth has been described in the marsupials and the guinea-pig. In Ornithorhynchus, Poulton has mentioned the presence of epithelial nodules in connection with the developing teeth. In this same form, Wilson and Hill describe two epithelial pearls which overlie the cusps of the first molar in each jaw, and three overlying the cusps of the second molar in each jaw. Two of these nodules are described as having a connective tissue pulp, inclosed by a distinct ring of dentine outside of which is a layer of columnar cells representing the inner enamel epithelium, and

finally on the periphery, a connective tissue capsule. The rest of these nodules is entirely epithelial, having a "central cellular core inclosed by concentrically arranged flattened cells forming a compact zone." The authors interpret these 'nodules' as the vestigial remains of an earlier dentition. They arise "by structural differentiation of the labial aspect of the enamel organ, and occur only in those regions of the lamina which constitute the enamel organs of the future teeth." Over lying teeth which are about to erupt, Wilson and Hill also found in Perameles sporadic cases of nodules composed of concentrically arranged epithelial cells.

Marrett Tims describes similar structures overlying the first premaxilla and second molar of the guinea-pig, and interprets them as representing the last vestigial remains of the milk dentition. A spherical body composed of "concentrically arranged epithelial cells" has been found by Tims in connection with the last premolar of the dog, and by Woodward in connection with the last premolar of Gymnura. Tims believes there is thus established a graded series in degenerating teeth. He recalls the fact that Woodward has figured a "calcified vestigial incisor in the mouse which, in cross section, appears as a narrow loop of dentine forming three-quarters of a circle and bordered by cells of the enamel organ." Tims says that it is not hard to imagine that a still further stage of degeneration would give rise to a stage like that seen in the dog, guinea-pig, and Gymnura.

In the lower jaw of the edentate, Manis javanica, Tims has also described and figured a downward growth of the oral epithelium which he has interpreted as representing a tooth vestige. In some sections this downgrowth lies in the underlying tissue unconnected with the oral epithelium. In the figures these structures look exactly like the first stages in the formation of the cysts in the upper jaw of Dasypus.

I believe that these cysts occurring in the upper jaw of D. novemcinctus represent tooth vestiges. My reasons are as follows:

1. They always occur in the same place,—the extreme posterior end of the premaxilla. They are found in the same relation to the dental ridge as are the germs of the functional back teeth.

2. Nowhere else in the jaw have I found similar structures.

3. Their number is fairly constant, ranging from three to five.

4. They are similar in structure to the so-called 'epithelial pearls' whose relation to tooth formation has been recognized.

5. Before transformation into horny material, their structure is exactly like that of the 'nodules' and 'concentric epithelial bodies' which occur in Perameles, Ornithorhynchus, and the Gymnura, and which are evidently vestigial teeth.

In none of the cases where epithelial pearls have been interpreted to represent vestigial teeth has their transformation into horny material been described. If later stages of the embryos of Ornithorhynchus, Perameles, and the Gymnura were studied, it is very probable that horny cysts would be found. In Dasypus, these pearls have so far lost their capacity to form tooth germs that, inside the substance of the jaw, they behave just as they would had they remained on the outside. Downward growth into the underlying tissue seems to be the only action which is reminiscent of their former behavior as enamel organs. As I have mentioned above, Bland-Sutton has found all gradations between epithelial pearls, horny cysts and enamel organs.

Since these structures always occur in the premaxilla, they may represent incisors. In this connection it is interesting to note that their maximum number is five, and that this is also the maximum number of vestigial incisors in the lower jaw. This raises the question as to where the missing canine is. I can only state that with the exception of the dental lamina which extends through this region in the early embryo, I have found no trace of tooth germs between the first of the functional back teeth and the last of the vestigial front teeth. For this reason, I think that the last of these vestigial front teeth may represent the canine.

G. SUMMARY

1. In the embryonic lower jaw, the anlagen of either thirteen or fourteen teeth arise.

2. The last eight of these teeth germs become back teeth. The anterior tooth germs do not develop into functional teeth.

3. Of the functional back teeth, the eighth has no predecessor in the milk dentition. The other seven are replaced by permanent successors.

4. The functional back teeth of both dentitions develop normally. They are rootless, grow from persistent pulps, possess normal tubular dentine, and a thin layer of enamel.

5. The first, second, and eighth back teeth are one-cusped; the others are bicuspid with a higher lingual and a lower labial cusp. The first tooth is always one-cusped; the second and eighth are bicuspid at first, but later become one-cusped through the upward growth and obliteration of the original groove between the cusps. In the seventh tooth there appears a tendency to suppression of the labial cusp.

6. Of the non-functional front teeth, the sixth and last is the only one which erupts, and it is shed soon after eruption. The other front teeth are absorbed.

7. The sixth is the largest of the front teeth, has the widest deposit of enamel and is the only front tooth in which all of the dentine is of the normal tubular variety.

8. The other front teeth become increasingly smaller from behind forwards and in somewhat over fifty per cent of cases, the first tooth is represented by a degenerating enamel organ. The relative thickness of enamel diminishes towards the anterior end of the jaw until in the first two teeth it is so thin that it can be definitely distinguished only with the aid of an oil immersion lens. In the anterior end of the jaw, there is also an increasing tendency towards the formation of cellular dentine, and the obliteration of the pulp cavity by the deposition of dentine.

9. The formation of secondary tooth buds in connection with these nonfunctional front teeth has not been conclusively demonstrated.

10. The dental formula of the lower jaw is probably M 1, Pm 7, C 1, I 6 or 5.

11. In the maxilla there arise the anlagen of eight functional back teeth whose structure and development is like that of the corresponding teeth of the lower jaw.

12. Between the first functional back tooth and the posterior end of the premaxilla, there exists an extensive diastema. The dental lamina extends through this region but degenerates without warning giving rise to tooth germs.

13. In the extreme posterior end of the premaxilla there occur from three to five upgrowths of the oral epithelium. These become detached from their place of origin and form cysts, the contents of which resemble an oral epithelium which has become horny like the peripheral layer of the stratum corneum. These cysts evidently represent tooth anlagen which have lost all likeness to enamel organs and behave as if they were still on the surface of the jaw.

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PLATES

ABBREVIATIONS

BV, Blood-vessel
C, Cartilage
CD, Calcified dentine
CT, Connective tissue
D, Dentine
DC, Dermal cyst
DF, Dentinal fibril
DP, Dental papilla
E, Enamel
EB, Epithelial bud
EO, Enamel organ
EP, Enamel pulp
FE, First formed enamel
HDC, Cornified dermal cyst
HSC, Cornified stratum corneum
IEP, Inner enamel epithelium
LA, Labial side

LE, Last formed enamel
LI, Lingual side
M, Mesoderm
OE, Oral epithelium
OEP, Outer enamel epithelium
PT, Permanent tooth bud
PC, Pulp cavity
RDL, Remains of dental lamina
REO, Remains of enamel organ
S, Shrinkage space
SC, Stratum corneum
SG, Stratum germinativum
SI, Stratum intermedium
SR, Stellate reticulum
T, Tomes processes
UD, Uncalcified dentine

7   Section through a back tooth at birth showing relative thickness of enamel the deposition of which has been completed at this stage.   The germ of the permanent tooth appears as a narrow cord of cells at the lingual side of its milk predecessor.   × 70.

8   Section through germ of the eighth back tooth at birth (108 mm.).   × 380.

9   Section at birth through the cord of cells which represents the germ of the permanent tooth.   × 1800.

10 Section through enamel and enamel organ of permanent tooth a few months after birth.   × 560.
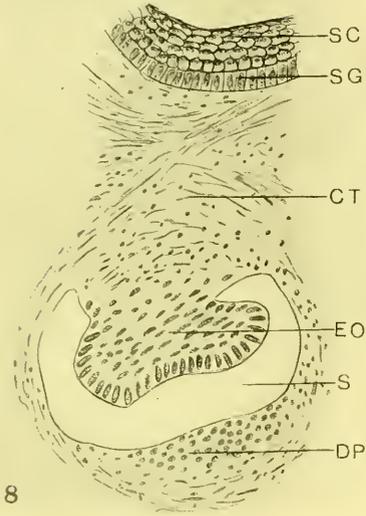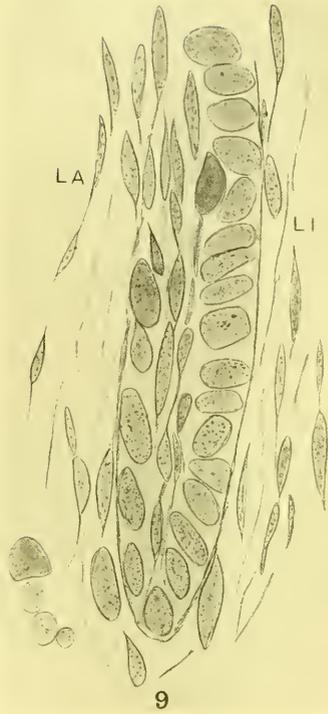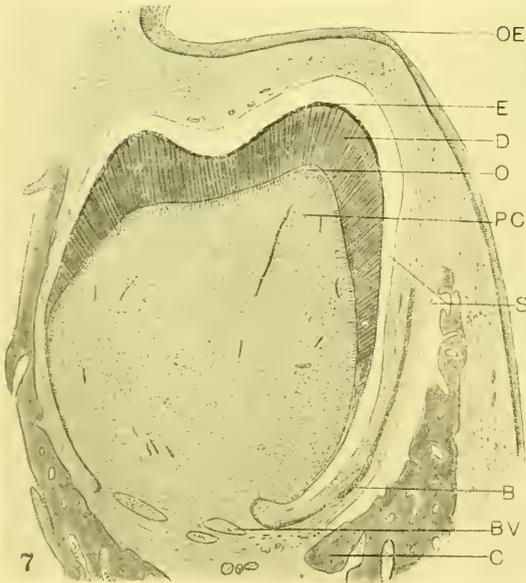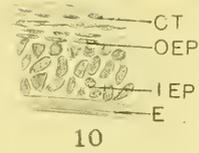
11   Section through tooth germ in the anterior part of the jaw of a 30 mm. embryo showing the wide separation from the oral epithelium.   Compare this figure with figure 1. $\times$ 560.

12   Section through the tooth germ of the sixth front tooth of a 53 mm. embryo.   The parts of the enamel organ are at the height of their development but the enamel pulp bears no resemblance to a stellate reticulum. $\times$ 560.

13   Section through the sixth front tooth of a 78 mm. embryo. $\times$ 200.

14   Section through the sixth front tooth at birth (108 mm.). $\times$ 200.
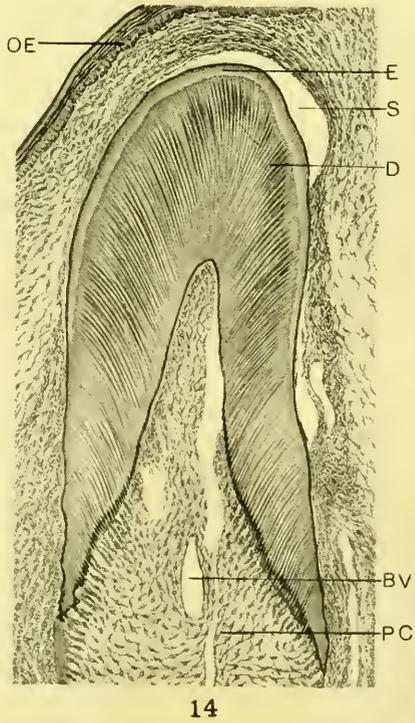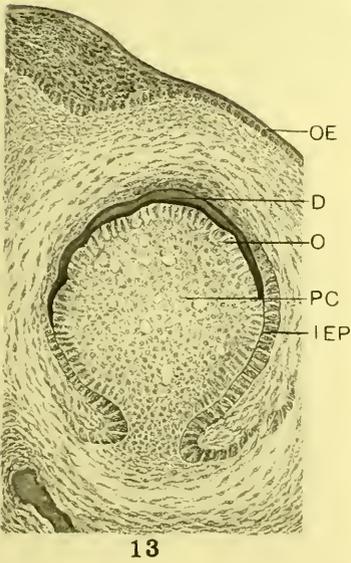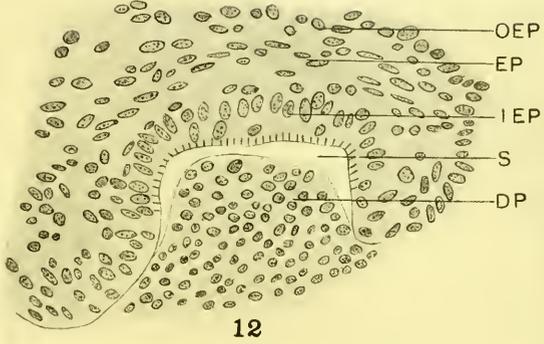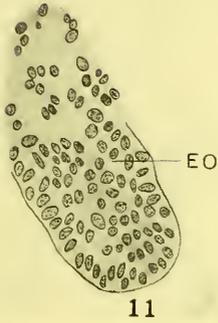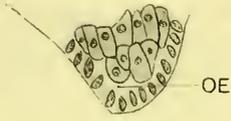
15 Section through the oral epithelium in the posterior part of the premaxilla region of a 65 mm. embryo showing a bud-like upgrowth of the epithelium.   × 560.

16 Section through posterior part of premaxilla region of same embryo showing a detached epithelial bud or cyst.   × 560.

17 Section through dermal cyst in an 82 mm. embryo.   × 400.

18 Section through dermal cyst at birth (108 mm.).   The central portion of the cyst is cornified like the peripheral layer of the oral epithelium.   × 400.

SUBJECT AND AUTHOR INDEX