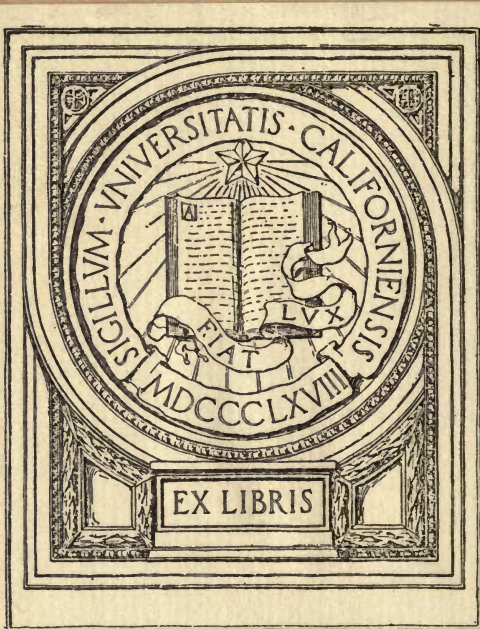# Development of the Nine-Banded Armadillo from the Primitive Streak Stage to Birth: With Especial Reference to the Question of Specific Polyembryony

H. H. NEWMAN AND J. THOMAS PATTERSON

From the Zoölogical Laboratory, University of Texas

FIFTEEN TEXT FIGURES AND NINE PLATES

THE DEVELOPMENT OF THE NINE-BANDED ARMADILLO FROM THE PRIMITIVE STREAK STAGE TO BIRTH; WITH ESPECIAL REFERENCE TO THE QUESTION OF SPECIFIC POLYEMBRYONY[1]

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I. Introduction

A. Review of the Literature

It is not our present purpose to attempt any comprehensive review of the literature dealing with the development of the Edentata, nor even of that treating especially of the armadillos. It seems advisable, rather, to limit our survey to those contributions, a knowledge of which is essential to an understanding of the problem of specific polyembryony.

That certain species of armadillos bring forth at a birth young all of one sex has been known for over a century. According to Azara,[2] a writer of the eighteenth century, the natives of Paraguay and of the Argentine Republic knew that this was true for the Mulita (Tatu hybridum). Any observant hunter, who had been fortunate enough to capture a litter or two of young animals in a burrow with the mother, might readily have noted such a unique state of affairs, for the sexes are easily distinguishable.

In the latter part of the nineteenth century Herman von Jhering, ('85 and '86), met with similar statements on the part of the natives of Brazil and was sufficiently interested to attempt a scientific confirmation of what had been until then merely an interesting piece of folklore. Two pregnant females came under his observation, the uterus of each of which contained eight male foetuses, all in exactly the same stage of development. Each foetus was described as having its own separate amnion; but all were surrounded by a common chorion.

These conditions were interpreted in a subsequent paper by the same author as indicating the origin of the several embryos from a single fertilized egg, and it was further assumed from the facts in hand that the splitting of the original single germ into separate embryonic primordia occurred at some period after fertilization. Von Jhering apparently saw nothing more fundamental in this situation than the discovery of a new type of animal reproduction to which he gave the name "temnogenesis." Its bearings on the problems of sex determination and of heredity

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[2] Referred to by von Jhering.

were not appreciated. To him however belongs the credit of having discovered specific polyembryony in the Mulita.

No attempt was made to secure evidence, either internal or external, of the validity of von Jhering's suggestion until Rosner, ('01), took up the subject in connection with his studies of human monochorial twins. On the basis of a histological examination of the ovaries of one pregnant female of the South American nine-banded armadillo he attempted completely to discredit the idea that the several embryos of a litter arise from a single fertilized ovum. Since his observations strike at the very foundations of the question of polyembryony in the armadillos it seems necessary to review his work in some detail.

The genitalia of two pregnant females were sent to him by von Jhering, and an examination showed that the ovaries of only one specimen were sufficiently well preserved to admit of histological examination. Sections of the other pair of ovaries showed that a large percentage of follicles contained more than one egg. There were in all 52 large follicles: 11 with 2 eggs, 7 with 3, 2 with 4, 1 with 5, and 1 with 7. The two largest follicles contained four eggs, exactly the number necessary to produce the four embryos habitually brought forth in a litter of this species. Since the youngest follicles never contained more than one egg the conditions seen in the older ones must have resulted from secondary fusions of adjacent follicular walls, which subsequently disappeared in such a way as to form a common cavity. The author's figures are evidently accurate representations of actual observations and are calculated to convince the reader. Especially striking is the figure of a reconstruction of a series of sections through a large pluriovular follicle in which each of the eggs has its own thick coating of discus proligerus cells.

Rosner believes that the observed condition of four embryos surrounded by a common chorion is to be explained by the following sequence of events: four adjacent follicles fuse in such a way that four eggs are thrown into a single cavity; on the rupture of this compound follicle the four eggs are discharged simultaneously, descend the fallopian tube held together in a mass by means of their discus proligerus cells, become fertilized, undergo

cleavage and come to a common point of attachment in the uterus; subsequently the contiguous walls of the four blastocysts atrophy and a single vesicular chorion is produced.

Were Rosner's observations a record of the normal conditions in the armadillo ovary the question of specific polyembryony would assume an aspect entirely different from that suggested by von Jhering, and we would need to seek no further for an explanation of the observed conditions. The observation that all the embryos in a litter are of the same sex was summarily dealt with by Rosner who considered it as interesting but in no way connected with the presence of a common chorion. Fortunately however there is now every reason to believe that Rosner's material was pathological or otherwise exceptional, for no subsequent investigator has been able to find in the armadillo ovary conditions such as he described.

Cuenot, ('03), while engaged in the study of the problem of the determination of sex, examined the ovaries of one pregnant and of one virgin female of the species investigated by Rosner. In the ovaries of the pregnant specimen there occurred only one follicle of the pluriovular type and this contained only two small, rather abnormal ova. Out of 119 follicles in the ovaries of the virgin female however three contained two or three eggs, but none was found with the number requisite to give rise to the number of young habitually born in a litter.

Until quite recently no further progress was made toward the solution of the problem. In 1909, however, there appeared almost simultaneously and quite independently, two contributions to the subject, one by Fernandez, ('09), on the Mulita (Tatu hybridum), and the other a preliminary report by the present writers, ('09), on the North American armadillo (T. novemcinctum). The two species evidently agree very closely in many of the more fundamental details of development but differ sufficiently to make it both interesting and valuable, from the comparative standpoint, to have the developmental history of both species worked out in the fullest detail.

Fernandez presents somewhat detailed descriptions of seven rather early embryonic stages and enters upon a brief discussion

of some of the more important questions involved. He was especially fortunate in securing in a good state of preservation two very young embryonic vesicles in which the demarkation of the several embryonic primordia had not yet manifested itself. For the equivalent of this stage we have looked in vain and hence, for the present at any rate, are compelled to rely on Fernandez's description for an explanation of our own earliest stages. Since it is necessary constantly to refer to Fernandez's work in the body of the text no further comment of an introductory character is needed here.

At this point it becomes necessary to refer to our own preliminary report in order to correct the description of fig. 3 in that paper. The specimen there figured was presented to us with the statement that it was intact in every respect, except that the uterus and the contained vesicle had been slit open along the mid-ventral line. On the basis of this statement, together with a study of the external features, we reconstructed the vesicle *in situ*. Our subsequent investigations of fresh specimens has led us to suspect that what we took to be a young vesicle was in reality only the villous portion of a somewhat later stage.

B. *Material and Methods*

During the past two years we have had the opportunity of examining 137 females of the native armadillo, together with a considerable number of males. During the breeding season hunters employed to collect material for us covered a wide range of territory in south-central Texas. These men were frequently obliged to haul the living animals through rough country for distances of fifty miles or more in order to reach an express office whence they could be shipped to our laboratories. As a rule a number of days elapsed between the capture of the animals and their arrival in Austin. This delay would serve in part to explain our ill success in securing the earliest embryonic stages. In order to obtain a complete series we believe it will be necessary either to breed the animals in captivity or to accompany the hunters on their expeditions so as to lose no time in examining freshly

fertilized females. Although we fully expect to secure the earliest
stages in the course of time it seems inadvisable for us to post-
pone the publication of the results thus far obtained, results suffi-
ciently clean cut in themselves to form the basis of a self-consis-
tent and fairly well rounded embryological account.

At present we have in our possession seventy embryonic vesicles
comprising a close series of stages ranging from the primitive streak
stage to birth.

Little need be said about the methods employed. To each ani-
mal that reached the laboratory was given a number and a page
in a ledger where all facts that might be of interest were recorded.
In case the carcase was to be thrown away complete records of
all data that might be useful in the future were kept. The ovaries
of the majority of the females were fixed in the standard cytologi-
cal fluids. Every part taken from a given specimen was numbered
accordingly. Much of the data thus gathered proved useful
during the course of the work and we have no doubt that all of it
will ultimately serve to throw light on future investigations.

C. Purpose and Scope of the Present Paper

In this our second contribution to the developmental history of
the armadillos the main purpose in view is to establish the fact of
specific polyembryony and thus to clear the way for future in-
vestigation. A more or less tentative explanation of its causes
and of the conditions and relations that result from it is hazarded
on the strength of the evidence now in hand, which is internal in
contradistinction to that derived from an examination of the ovar-
ies and testes, no detailed discussion of which is attempted at
present.

Although the question of polyembryony is the central problem
it is impossible to treat of it as an isolated phenomenon for the
reason that many curious developmental processes are intimately
associated with it. The history of the amnion and of the placenta,
for example, would be indecipherable apart from the fact of poly-
embryony, and the inter-relationships of the embryos admit of
a rational explanation on no other basis. The associated phenome-

non of germ layer inversion is also (indissolubly) bound up with poly-embryony and in turn involves many peculiar and interesting relations.

Any adequate treatment of the principal problem will therefore necessitate the presentation of a somewhat complex array of facts whose combined verdict will, we trust, establish our main contention.

Except in the case of the two earliest stages described no attempt is made to present a detailed account of the organogeny of the species. No doubt such a study would reveal many facts of interest to the specialist in mammalian embryology, but would serve only to cloud the main issue with obscuring details.

II. The Female Genitalia

The uterus is simple and not unlike that of the primates in form. In the non-pregnant condition it varies somewhat in size and shape according to the previous history of the individual. In old females that have produced a number of litters the organ though non-pregnant may be distended to several times its normal size, often leading the observer into the vain hope of finding the earliest stages. The uterus of the virgin adult presents a less modified condition and will furnish a basis for the accompanying detailed description.

The average dimensions of the non-pregnant uterus are as follows: 13 mm. from the tip of the fundus to the junction of the cervix with the vagina, 15 mm. between the points of entrance of the two fallopian tubes, and 10 mm. deep dorso-ventrally. Viewed from the dorsal aspect the uterus appears to be broadly kite-shaped (fig. 7) with the posterior angle blending into the vagina. The fallopian tubes are approximately straight where they enter the uterus, but near the ovaries are strongly convoluted, each ending in a hood-shaped fimbriated infundibulum, which, with the aid of a posteriorly directed flap of the broad ligament, covers a large part of the ovary and thus renders the escape of the ovum into the body-cavity well-nigh impossible. The points

of entrance of the fallopian tubes are about equadistant from the tip of the fundus and the vagina, thus rendering the cavity of the uterine body much larger as compared with that of the cervix than is the case in the human uterus, where the tubes enter practically at the distal end of the organ.

The ovaries are kidney-shaped having the convex side directed anteriorly, with reference to the axis of the animal. In virgin females the two ovaries are approximately equal in size, but in individuals that are or have been recently pregnant there is always a considerable difference in the size of the two ovaries. The larger one may be two or three times as large as the smaller, and this greater size is invariably due to the presence of a single enormous corpus luteum, the actual bulk of which may be much greater than that of the remaining ovarian tissue. There are found notinfrequently smaller bodies (resembling in histológical appearance the large corpus luteum) which are crowded to one end of the ovary and suggest by their shrunken and irregular form that they are either relics of a previous pregnancy or simply the lutea of ova which were never fertilized. It may be stated without hesitation however that *there is never more than one large and prominent corpus luteum in the ovaries of a pregnant female.*

The mucosa of the uterus is undoubtedly deciduate in character, as may be seen in the illustration of a section taken from a series cut through a pregnant uterus and its contents (fig. 1). Even at the comparatively early period represented it can readily be seen that the mucosa is separated from the outer layers of the uterus by a lymph space of considerable magnitude.

Since the young embryonic vesicle always gains attachment to the mucosa near the tip of the fundus it is not a difficult matter to orient it with reference to the uterine axis. It will be found convenient to refer to the fundus and cervix ends of the vesicle, the former being the original attached and the latter the original free end. The axis of each embryo is also related to that of the uterus, in that its anterior extremity is directed towards the cervix end of the vesicle, except in advanced conditions when the length of the umbilical cord occasionally permits an embryo to reverse its position within its amniotic sac.

The pregnant uterus assumes a variety of shapes in different individuals. At approximately the same period of pregnancy it may be either elongated or comparatively broad, either blunt or pointed at one or both ends, and either simple or clearly bilobed dorso-ventrally at the fundus end (figs. 42 and 43). These various forms are not due to the position or arrangement of the foetuses, which in this respect are practically constant, but probably to individual variation influenced by the previous functional history of the organ.

III. Number, Arrangement and Sex of the Embryos

A. Number of Embryos

In sixty-five out of seventy cases there were four normal embryos in a vesicle. It may be assumed then that four is typical for the species. Three atypical conditions occurred which may be listed as follows:

1. Vesicles containing five normal embryos (three cases, nos. 28, 91, 108).

2. Vesicle containing three normal embryos each measuring 15 mm. and one decidedly abnormal embryo 7 mm. in length (no. 57). No doubt this vesicle was destined to produce a three-embryo litter.

3. A case of twins (no. 137). These were born in captivity. A very careful examination of the uterus and intestines of the mother convinced us that there were no other young born. This may have been a case somewhat like the preceding except that two embryos degenerated instead of one.

There appear not infrequently in otherwise normal embryonic vesicles small amniotic sacs that usually contain the more or less completely degenerated remains of what may once have been extra embryos. In one case (no. 108), a vesicle with five normal 'embryos, such a sac appeared, which, if truly the representative of an extra embryo, would furnish an example of a six-

embryo vesicle. In another case (no. 17), which is peculiar in several other respects, there occurred a small empty amniotic sac fused firmly to the wall of the Träger and connected with the amniotic sac of a normal embryo by means of an amniotic canal similar to those of the other embryos. In still another case (no. 9) a fairly large sac in the Träger region was connected by means of a perfect amniotic canal with that of a normal embryo (fig. 44). There is little doubt but that these sacs represent the remnants of supernumerary embryos and as such are the equivalent of those described by von Jhering and Fernandez.

It is interesting to note in this connection that Tatu novemcinctum shows a stronger tendency toward stability in the number of foetuses in a litter than does T. hybridum. There is evident, however, in the latter species, a tendency to produce eight young in a litter, just twice the number typical for our species. The numbers of individuals in a litter ranges, however, from seven to twelve.

B. Arrangement of Embryos

In order to clear the way for the description of the early embryonic conditions it should provisionally be pointed out that the four embryos of this species are arranged in pairs, one pair to each lateral half of the uterus. The upper embryo of the left hand pair usually occupies the dorsal amniotic quadrant and is therefore referred to as the "dorsal embryo" (no. III). The lower embryo of the left hand side occupies the left lateral amniotic quadrant and is referred to as the "left lateral" embryo (no. IV). The lower embryo of the right hand pair occupies the ventral amniotic quadrant and is the "ventral embryo" (no. I), while its mate, occupying the right lateral quadrant is spoken of as the "right lateral" embryo (no. II). Nos. I and II constitute the right hand pair and nos. III and IV the left.

The orientation of the vesicle in the uterus and the arrangement of the four embryos with reference to the vesicle and to one another is rather precise, so that a plane running from the mid-dorsal to the mid-ventral line of the uterus would divide
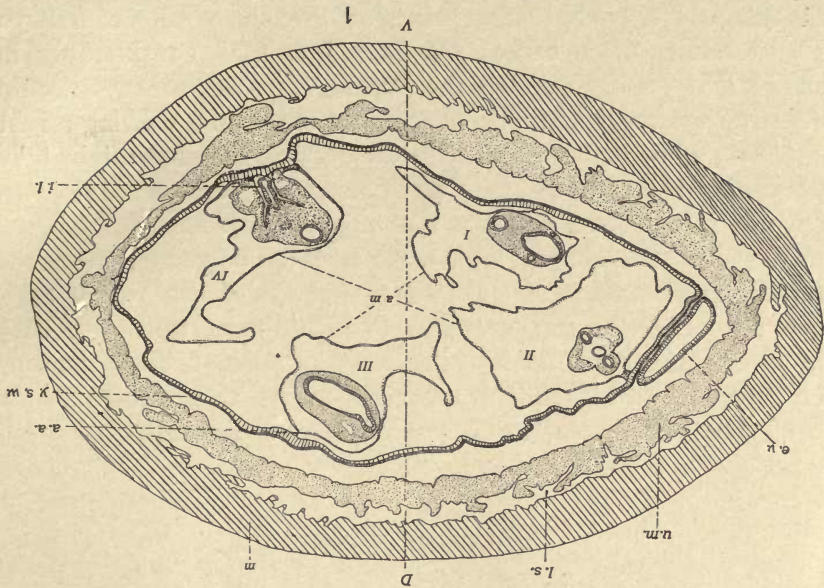
FIG. 1. Outline camera drawing of a transverse section through a pregnant uterus measuring about 15 mm. long by 14 mm. wide. Line D -V is drawn from the points lying at the middle of the dorsal and ventral sides of the vesicle. It divides the section of the vesicle into halves. Embryos I and II lie in the left hand half, and II and IV in the right hand half. *a.a.*, line of attachment of the amnion to the vesicle; *e.v.*, a small extra chorionic vesicle, which is not fused with the larger one; *i.l.*, intestinal loop; *l.s.*, lymph sinus between the wall of the vesicle and the uterine mucosa, *um.* × 9.

the two pairs of embryos and their placental areas from each other. There may be a secondary shifting of the positions of the various amniotic sacs, so that in the definitive condition one may find the upper embryo of the right hand pair occupying the dorsal position, which in the great majority of cases is occupied by the upper left hand embryo. Such a shifting might easily occur at any time before the walls of the various amnia fuse firmly with the chorion, a process that does not occur until a late period of gestation. Previous to this time each amnion is attached to the chorion only along a meridional line, an attachment that would permit the whole sac to swing almost as readily to one side as to

the other.   Reference to fig. 1 will show that the amnion of embryo II, especially after the amnia have increased considerably in size, might readily overlap the line D-V, so its embryo would occupy the dorsal amniotic quadrant.   The same shifting might equally well occur on the ventral side.   Such shiftings might take place however without affecting in any way the point of the embryonic attachment, which is immediately adjacent to the original amniotic attachment (fig. 1, *a.a.*).   Such departures from the typical arrangement of embryos in the vesicle are rather rare, and are not to be considered as of prime importance, for they in no way affect the pairing of embryos, a relationship depending on the point of attachment of the latter which is equivalent to their point of origin.   The significance of this arrangement is discussed in a subsequent chapter.

C. Sex of Embryos

In thirty-eight embryonic vesicles the foetuses are sufficiently advanced to permit of the accurate determination of their sex. There is no exception to the rule that *all embryos in a vesicle are of the same sex.*

Although the armadillo hunters claim that males are considerably more numerous than females we find no inequality of sexes in the sets of embryos in our collection, exactly half of which are male and half female.   In the small collection of nine advanced sets of mulita embryos Fernandez found that six were female and three male.   On this basis he proceeds to discuss the significance of the apparent disproportion of sexes in the species.   No doubt a larger collection of embryonic sets would have shown no such disproportion, for in our earlier survey of the subject of sex distribution we found a much larger proportion of males.

IV. The Early Embryology

In the development of the nine-banded armadillo we find that striking peculiarity, met with in the rodents, of germ-layer inversion. In the case of the armadillo the inversion is intimately bound up with the formation of the four embryos, and without it the mechanics of specific polyembryony, as found here, would be inexplicable. The possession of a common amnion by the embryos at an early stage could only occur as a sequence to inversion, and strongly suggests that the embryos are the product of a single fertilized egg.

In the present description of Tatu novemcinctum we shall begin with the primitive streak stage, and leave out of account the younger embryos (except for a brief reference to the work of Fernandez) until we shall have secured a series covering that important period. In dealing with the following stages considerable emphasis is placed upon the embryological details, and especially upon the relations existing between the embryos. This is done because these stages furnish the strongest internal evidence for polyembryony that has been brought forward.

A. The Earliest Stages of Fernandez

It will be necessary to refer to the work of Fernandez, especially to the part in which he describes his youngest two stages; because they hold the key not only to the morphology of the older embryos of Tatu hybridum, but also, we believe, to that of the stage of T. novemcinctum which we are about to consider.

Fernandez secured two specimens of his earliest stage, and the one he describes in detail was cut longitudinally into twenty-three sections (10 microns thick). It was found attached to the mucus membrane at the bottom of a fold at the fundus end of the uterus.

Fernandez correctly interprets the condition presented in this early stage as one having been brought about through the process of germ-layer inversion, and compares the vesicle to corre-

sponding stages of the rat and the mouse, described respectively by Selenka, '84, fig. 29, Taf. XIV., and Melissinos, '07, figs. 38 and 39 Taf. XXXIV. He thus finds the vesicle composed of three sacs lying one within the other: the innermost one is the ectoderm, the middle the entoderm, and the outer the tropho-blast (hinfälligen Ectoderm), which at the proximal or attached end of the vesicle is differentiating into the Träger. The simil-arity between the vesicle of Fernandez and those figured by Melis-sinos (his figs. 38 and 39) is particularly striking, though, as he points out, there are several differences. In the first place, the mesoderm is not yet formed and the so-called Träger cavity scarce-ly can be regarded as homologous with that of the mouse. In the second place, the parietal layer of the yolk-sac entoderm is not complete, but is wanting in the distal portion of the trophoblast. If, however, we may be allowed to make a suggestion based on a study of his photograph (fig. 6, Taf. XIX), what appear to be scattering cells lying along the inner surface of the distal tropho-blast might well be interpreted as representing the remains of the parietal layer of the yolk-sac. This would make this early stage of the Mulita very closely resemble the corresponding stages of several other forms, as illustrated in the figures of such investi-gators as Selenka ('84), Robinson ('92), Jenkinson ('00), and Mellissinos ('07).

The most interesting portion of this young vesicle of the Mulita is the inner sac, for it is the primordium out of which the ecto-derm of the several embryos later differentiates. Fernandez points out the significant fact that it gives no indication of being a multiple structure, such as one would expect to see if the vesicle were the product of the fusion of several eggs.

The second stage of Fernandez is decidedly more advanced than the preceding, and was found lying loose in the fundus end of the uterus. In the preserved condition it measured 3 mm. long by 2.3–2.5 mm. wide. The general condition of the germ layers in this vesicle is made clear in the slightly modified copy of his second text-figure (fig. 2). The figure, which is a diagram of a median longitudinal section passing through two embryos, is shaped like a horse shoe. The entire convex anterior and lateral
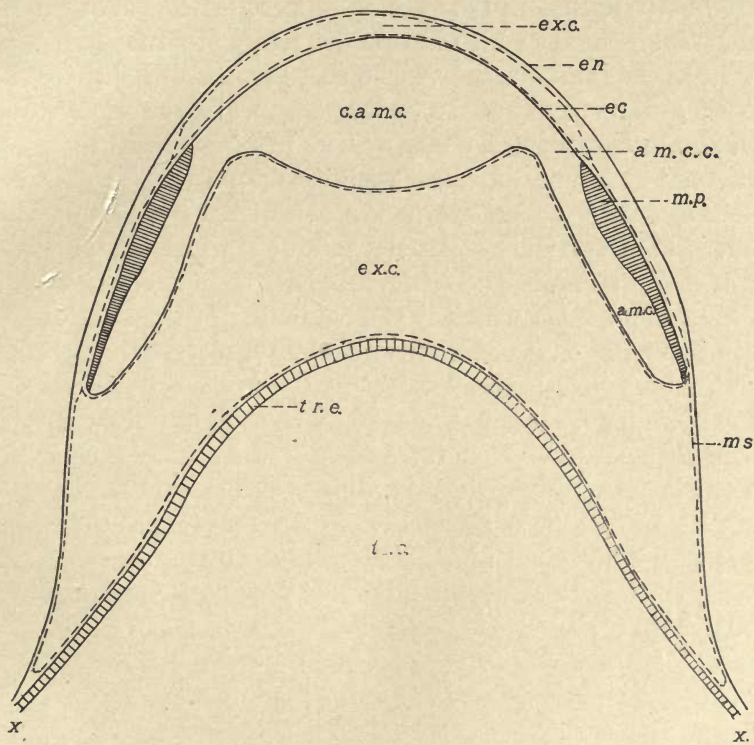
Fig. 2. A diagrammatic longitudinal section of an early stage of the Mulita. *ex.c.*, extraembryonic body cavity; *en.*, entoderm; *am.c.*, amniotic cavity of the embryo; *am.c.c.*, beginning of the amniotic connecting canal; *c.am.c.*, cavity of the common amnion; *ms.*, mesoderm; *m.p.*, medullary plate; *tr.c.*, Träger cavity; *tr.e.*, Träger epithelium; (slightly modified after Fernandez).

margins represent the entoderm of the inverted yolk-sac, while the concave posterior margin is covered with Träger epithelium. Between these two regions occurs a narrow zone where the vesicle was attached to the uterine wall (marked X).

The Träger cavity (*tr. c.*) is situated in the concave space roofed over by the Träger epithelium. While in some respects this cavity is comparable to that of the rodents, yet for the most part any such comparison would appear to be strained. The difficulty standing in the way of pointing out any true homologies, however,

must be attributed to the incompleteness of the history of these early stages—a fact which Fernandez freely admits.

Within the limits of the vesicle there are two distinct cavities: one the general cavity of the vesicle (*ex. c.*), and the other the common amniotic cavity (*c. am. c.*). The former is lined throughout with mesoderm, and the latter with ectoderm.

The embryos, which are in the medullary plate stage, lie in pocket-like diverticula from the lateral margins of the floor of the common amnion; and each embryo is connected with the latter by a short tube, which is the beginning of the amniotic connecting canal. The common amnion, together with its accompanying embryos, is the product of the inner ectodermal sac of the earlier stage. It is not at all easy to explain fully the manner in which the various structures presented in this vesicle develop out of the primordia of the preceding vesicle, although the history of several of them is self evident. To go from this to the succeeding stage is, however, an easy step, and we shall therefore pass directly to it as exemplified in our youngest vesicle of Tatu novemcinctum.

B. The Primitive Streak Stage

We were fortunate in being able to secure from the uterus the entire embryonic vesicle in practically a perfect state of preservation. The opportunity was thus afforded not only to make a detailed study of the relations existing between the different embryos but also to obtain a drawing of the vesicle as a semitransparent object (fig. 12). In the preserved condition it measured 7 mm. wide by 9 mm. long. It is slightly flattened dorso-ventrally but in general outline is shaped like an inverted balloon, with two lateral horn-like projections which fit into the openings of the fallopian tubes. These horns persist for a considerable time and are of great service in aiding one to maintain the correct orientation of the vesicle during its early development.

The surface of the vesicle presents two distinct regions, the lower of which fits into the fundus end of the uterus and is recognized as the Träger. It is therefore covered by Träger epithe-

lium.   At the extreme lower end there is a small cap-like area where the primitive attachment knots or cords of the Träger epithelium are beginning to disappear.   The other region occupies the upper two-thirds of the vesicle and differs from the preceding both in its greater transparency and in the complete absence of a trophoblast.   This region is the yolk-sac of the inverted type, and consequently is covered with the entoderm.   It is rather indistinctly divided into two portions: (1) the central zone occupied by the embryos and their vascular areas, and (2) the cap-like upper third in which the almost complete transparency is obstructed by the presence of the common amnion and its connecting canals.

Two of the embryos lie on the upper side (corresponding to the ventral side of the uterus) and two on the lower side of the vesicle. Each embryo is connected with the Träger region by a rather broad band, the belly-stalk, and is surrounded by an amnion. Since there is an inversion of germ layers, the embryos when viewed from the outside of the vesicle are seen from their ventral aspects; hence, the posterior portion of each amnion is invisible except as seen through the semi-transparent embryo.   Anteriorly, however, the lateral margins of the amnia are clearly distinguishable and are seen to pass forward as the tube-like, amniotic, connecting canals.   These lie on the inner or mesodermal surface of the yolk-sac, to which they are loosely attached, and in passing forward they converge and finally enter the common amnion. They do not communicate with this by four distinct openings, but by two, for just before reaching it, the canals belonging to the dorsal and left lateral embryos unite to form a single tube, as do also those belonging to the ventral and right lateral embryos. As will be pointed out in another section, this fusion of the canals is an indication of the pairing of the embryos since the union in each case is between individuals of a pair.

The common amnion at this stage is a comparatively small vesicle lying at the extreme cervix end of the vesicle.   The manner in which this condition has been evolved from that seen in the second stage of Fernandez is not difficult to figure out.   On the one hand, the cavity of the embryonic vesicle has undergone

an enormous extension, due in part to the natural growth of the vesicle and in part to the modification in the shape of the Träger wall, which has changed from concave to convex; on the other hand, the common amnion not only has failed to keep pace with this rapid expansion of the embryonic vesicle, but has actually ceased to grow at all, and is destined soon to degenerate and disappear. In the rapid growth of the embryonic vesicle the embryos gradually have been drawn away from the common amnion, and consequently their connections with it have been pulled out into the long, slender, tube-like canals.

The embryo viewed from the dorsal side shows the exact relations existing between it and the amnion (fig. 13). In general outline the embryo is slipper-shaped and throughout the greater part of its length the amnion conforms to this contour. Both anteriorly and posteriorly the amnion narrows down rapidly—in the former direction to produce the amniotic canal (*am. c. c.*) and in the latter to form the posterior amniotic process (*p. am. .p*), which ends blindly above the Träger. The level at which the amnion becomes narrower than the belly-stalk varies in different embryos. In the embryo in question it cuts in some distance posterior to the mouth of the allantois, but in other cases it may cut in at a level somewhat anterior to this point.

The entire embryo, from the anterior end of the medullary plate to the posterior tip of the amnion, measures 3.5 mm., but the embryo proper is only 2.5 mm. long. Running through the central part of the medullary plate is the elongated primitive streak, in which is a well developed primitive groove with a faintly defined primitive pit at its anterior end. The primitive streak is exactly 1 mm. long, and has at its anterior end a distinct head process measuring 0.28 mm.

The outline of the allantois is seen through the embryo, and begins a short distance back of the posterior end of the primitive streak and extends through the mesoderm of the belly-stalk, finally ending some distance anterior to the tip of the amnion. Fernandez does not describe the development of the allantois in the Mulita, and this stage is, of course, too far advanced to give any clue to the exact nature of its origin.

Lateral to the embryo is seen the beginning of the yolk-sac or vitelline circulation. At this time the blood islands are well developed and incipient blood vessels are represented by a network of anastomosing cords of mesoderm. About midway between any two contiguous embryos there is a band-like area extending from the Träger to the upper limit of the area vasculosa. The band represents the region where the boundaries of the vascular areas of adjacent embryos come together, and thus corresponds to the sinus terminalis of other forms, except that it is double in composition. At the anterior margin of the vascular area of each embryo the sinus terminalis tends to form the arc of a circle, a tendency which, if not inhibited by the crowding of four embryos, would result in the production of a circular sinus exactly as in other forms. As a result of this retardation by crowding the anterior margin of the vascular zone of the four embryos is in the form of a series of scallops.

For an appreciation of the condition of the germ layers it is necessary to turn to a study of representative sections. In the most typical of these, such as that taken through the primitive pit, the neural portion of the ectoderm is thick and has the general appearance of that of corresponding stages of other forms (fig. 19). The outer ends of the section curve decidedly upward, especially the one on the right, but for the most part this is due to the fact that the embryo conforms to the general curvature of vesicle. At the ends of the section the medullary plate turns upward to form the amniotic ectoderm, which is composed of a single layer of cells.

In the central part of the section the entoderm is composed of rather flattened cells, which, however, remain distinct from the overlying mesoderm. Beyond the limits of the primitive streak it becomes thicker and its cells are cuboidal in shape. It must be kept in mind that the entoderm actually forms the outer surface of this region of the vesicle; for the trophoblast has practically disappeared and there are found only a few of its cells scattered here and there along the outer surface of the entodermal layer.

The mesoderm is arising from the primitive streak region in the characteristic manner, and laterally it thins out and, at the point where the ectoderm turns up to give rise to the amnion, divides into two layers, one following closely the amniotic ectoderm and the other the yolk-sac entoderm.

Through the middle of the head process (fig. 18 *h. p.*) the entoderm at the center of the section is barely distinguishable from the mesoderm, and in many places the union of these two layers is very intimate. This must be looked upon however as a condition which is in all probability secondary. In the region of the head process proper the mesoderm cells are closely packed together, but are entirely separate from the neural plate.

Anterior to the head process the mesoderm rapidly thins out practically to a single layer of cells and is easily distinguishable from the entoderm (fig. 17).

Anterior to this section the mesoderm passes into a thickened region of the entoderm, which obviously has nothing to do with the mesoderm, but owes its existence to a proliferation of entoderm cells (fig. 16, *p. p. h.*). It was not detected in the whole mount preparations of the embryos, but its extent is easily determined by a study of sections. The thickening runs through the first five sections beginning with the anterior tip of the embryonic shield, and its width is equal to its length, and it therefore forms a circular plate about 45 microns in diameter. In every respect this circular spot corresponds to the "protochordal plate" of Hubrecht, ('08), who has laid especial emphasis upon it as a region where the entoderm is clearly a source of mesoderm formation. Whatever may be one's conviction regarding Professor Hubrecht's interpretation one can at least be certain that the thickening is purely of entodermal origin in this species. Our series is here too incomplete to permit of tracing out the history of the protochordal plate, and thus to see whether its definitive condition is simply that of mesoderm formation, or whether it contributes to the formation of the fore-gut or the oral plate.

It should be stated here that the protochordal plate at the stage under discussion thins out to a single layer towards its margin,

where it gradually passes into the surrounding entoderm. In many places the mesoderm cells are beginning to migrate in between the plate and the ectoderm, and especially is this true in the more anterior sections (fig. 21). In this section, which shows six of the mesodermal cells, the anterior limit of the protochordal plate is represented. A very short distance in front of this the sections pass through the amniotic canal (fig. 20), which is seen to be composed of two layers, a rather thick inner ectodermal layer, and a thin outer mesodermal layer. In some places the canal is loosely connected with the underlying mesoderm of the yolk-sac, but for the most part it merely lies in contact with the latter.

In sections lying posterior to the primitive pit there is nothing of especial note until we come to the region where the allantoic tube takes its origin. The mouth of the allantois is in the form of a deep groove traversing the ventral side of the anterior end of the belly-stalk (fig. 22, *al*). This is lined with an especially thick entoderm and gradually fades out anteriorly, but posteriorly suddenly narrows down to form the tube. The mesoderm of the belly-stalk appears to extend laterally to form the two wing-like processes, which are to be interpreted as representing cross section of the belly-stalk bands (*b. b.*). Externally these are covered with an epithelium, but within are composed of a loose mesodermal tissue in which run the umbilical blood vessels together with their accompanying sinuses. In section the posterior amniotic process is triangular in shape, and is not much more than half the width of the belly-stalk.

In sections taken through the posterior end of the embryo (fig. 23) the allantois is reduced to a slender tube, having a small lumen. The amnion is here triangular in cross section with the lower angle coming in close proximity to the allantoic entoderm. The mesoderm has much the same shape as in the preceding figure, but may be divided rather indistinctly into two portions: (1) the allantoic mesoderm which surrounds the entodermal tube, and has the cells compactly arranged; (2) the more distal wings or belly-stalk, bands through which the blood vessels run.

The semidiagrammatic longitudinal section of the primitive streak stage is shown in fig. 24, and in connection with what has

been said above concerning the transverse sections, this may be studied with profit. The entoderm in this section can be traced from the protochordal plate back along the entire length of the embryo. Throughout the greater part of its length it is composed of flattened cells, but near the posterior end of the primitive streak these cells become cuboidal, and in the region of the mouth of the allantoic tube (*al*) take on a columnar appearance. Posterior to the allantoic opening the yolk-sac passes back and ends abruptly at the margin of the Träger epithelium (*tr. e.*).

While the median section does not show the lateral belly-stalk bands which form the main connections between the embryo and the Träger, it does, however, bring out with clearness the union between these two as seen at the extreme tip of the embryo. This connection (*ms. co.*) is simply a backward and downward continuation of the allantoic mesoderm, which passes over into the general mesodermal lining of the Träger region.

C.   The Five to Seven Somite Stage

The general relations existing between the various parts of the embryonic vesicle in this stage closely resemble those of the primitive streak stage, but the vesicle is almost twice as large, measuring 15 mm. long by 14 mm. wide (fig. 14). Owing to this increase the horns are not only relatively but actually shorter than in the preceding stage. The Träger has undergone marked differentiation and shows a tendency to overgrow the yolk-sac region. The common amnion with its canals presents the same general features as before.

The most interesting changes have occurred in connection with the development of the embryos, and it is to these that we would direct attention. In the first place emphasis should be placed upon the fact that the embryos are not equally differentiated, for the dorsal and left lateral have each, five pairs of primitive segments while the ventral and right lateral embryos have seven. In other words, the individuals of the same pair are in the same stage of development.

In the five somite embryo (fig. 30) the neural folds have not yet coalesced to form the brain vesicle, and consequently the neural groove is open throughout its entire length. The posterior ends of the neural folds embrace the much reduced primitive streak. The embryos are bounded laterally by an area pellucida, which is rapidly being invaded by the blood cords.

In sharp contrast to this embryo is the individual from the other pair showing seven somites (fig. 31), and unless one were from the first aware that they were members of the same set of embryos, one would not so classify them. There are really only six and one-half somites in this embryo, for the most anterior or cephalic pair is connected with the head mesoderm and is somewhat smaller than the succeeding pairs (fig. 15). There is a slight indication of an eighth pair being cut off from the anterior end of the unsegmented paraxial mesoblast.

The amnion has undergone several marked changes, chief among which are (1) its enlargement in the cephalic region of the embryo and (2) its reduction in width at the level of the distal part of the belly-stalk. In this stage the neural folds have risen up and coalesced to form a portion of the neural tube. The point where the fusion first occurs is at the level of the mid-brain region, and from this place it progresses both backwards and forwards. The anterior progress of the union, however, takes place rather slowly and the final closing on the under side of the fore-brain to form the neuropore does not occur until a period much later than this.

At the posterior end of the diverging folds the reduced primitive streak is seen as a broad plate, which in the mid-ventral region is slightly concave, and by transmitted light appears to be decidedly thicker than the lateral portions. The notochord is seen to arise from the anterior end of the primitive streak and to extend forward between the folds. At the point of origin of the notochord the primitive streak is unusually thick, forming a distinct primitive knot, just back of which is the suggestion of a primitive pit. At the posterior end of the primitive streak the entodermal allantois is faintly visible. It extends backward lying beneath the floor of the posterior amniotic process, and falls far short of reaching the tip of the latter.

The belly-stalk now shows a tendency to form into two bands at the proximal or attached end. Each band later carries an umbilical artery and vein from the placental disc to the embryo, that is, they form the attachment of the umbilical cord to the wall of the vesicle. The anterior margins of the bands are turned up to form scroll-like structures beyond which the scale-like villi of the Träger are beginning to extend out over the yolk-sac (fig. 15 *s. v.*).

There is yet to be considered the yolk sac circulation. This consists of a net work of anastomosing mesodermal cords, which in section are seen to be composed of a central mass of incipient blood cells, surrounded on the upper side by an attenuated layer of mesoderm and on the lower by the entoderm (fig. 8, *b. c.*). These cords do not become hollowed out even at a much later period than this. Indeed it is doubtful whether they ever become functional blood vessels.

In considering the details of structure we shall confine our accounts to a brief description of a series of transverse sections of the five somite embryo, and to the median longitudinal section of a seven somite embryo.

In the region of the neural fold the neural groove has become greatly deepened to form the first rudiment of the brain vesicle (fig. 26, *n. g.*), and the lateral margins of the medullary plate have become tucked in beneath, thus forming a bay on each side that is at once recognized as the lateral extensions of the headfold (*h. f.*). In consequence of this folding the extreme lateral portions of the amniotic cavity have had the marginal parts of the medullary plate withdrawn from them, with the result that the walls of the amnion have more or less collapsed, obliterating the cavity. In all probability the obliteration is an artifact, due to the rupture of the amniotic canals and the consequent escape of the amniotic fluid.

In the central region the entoderm has undergone a transformation to produce the notochord (*n. ch.*) which consists of a row of columnar cells. Already the entoderm shows signs of beginning to grow beneath the notochord, so that this structure will soon be cut off from the archenteron. The primordia of the pharyn-

geal pouches (*ph. p.*) are seen as bays of entoderm lying on each side of the neural tube.

The mesoderm in this region is in two rather distinct forms; the outer portion is epithelial in character and conforms to the general contour of the entire surface of the section; and the other part is composed of mesenchyme and lies to each side of the imperfectly formed brain vesicle, and consists of scattering stellate cells.

The medullary plate gradually grows narrower as one passes backward until the region of the somites is reached, where its width is about one-third that of the entire embryo. The margins of the entoderm have almost grown together beneath the notochord. The mesoblastic somites are partly constricted off from the lateral plates, which are undergoing the process of splitting into the somatic and splanchnic layers, between which is the weakly developed coelome.

In the region of the proximal part of the allantois (fig. 28) the belly-stalk bands are very much folded, having their outer margins turned up to form the scrolls that were noted in fig. 15. The umbilical blood vessels in the bands are well organized and are lined with an endothelium. The only other structure worthy of special mention is the posterior amniotic process which is reduced to a small flat tube.

The final section of this series to be considered here is one taken through the posterior end of the amnion (fig. 29). The amnion and median posterior portions of the belly-stalk bands are connected by a rather slender stalk with the Träger (*ms. co.*). The exact nature of the Träger will be considered in another section, and it remains here merely to point out that the original primitive knots are being rapidly transformed into villi.

The longitudinal section of the seven somite embryo (fig. 25) should be compared with that of the primitive streak stage, in order to bring out the most significant changes occuring in development. The notochord lies exposed throughout the greater part of its length, but at each end it is covered beneath with the entoderm. At the posterior end, where the notochord is covered over, the entoderm is seen to turn back on itself for a short dis-

tance (fig. 25, *en'*). This is doubtless only an expression of the same process noted in the study of cross section, in which it was seen that the entoderm was growing in beneath the notochord.

The primitive streak has become greatly reduced, due to its transformation into the embryo. The final change to which we would call attention is seen in the great reduction in the length of the allantoic entoderm (*al*). It is now not more than one-half of its former length, and is soon destined completely to disappear.

V. History of the Placenta

Certain isolated stages in the development of the placenta have been described for at least three species of armadillo.

Kölliker ('76), Milne-Edwards ('78), and Duges ('79–'80), successively described the placental conditions seen in rather advanced vesicles of the South American nine-banded armadillo. Of these accounts that of Milne-Edwards appears to be the most detailed. The embryonic vesicle is described as being a pear-shaped body covered with a chorion, the proximal and distal parts of which were thin and membranous, while the middle part formed a thick, vascular, four-scalloped ring, composed of four fused placentae.

A stage similar to that just cited was recently described in somewhat greater detail by the present writers, ('09), and illustrated with two diagrammatic figures. This description of the North American variety of the species seems to agree closely with that of the South American variety as given by the authors just referred to. No doubt we have essentially the same species on both continents.

The only other reference to the placentation of Tatu novemcinctum is that of Lane ('09), who described in some detail the afterbirth of a specimen sent to him from central Texas.

A more comprehensive account of placental conditions is found for Tatu hybridum. Von Ihering states with reference to an advanced stage of placentation, that there is a zonary placenta which has nothing in common with that of the carnivora, but must be considered as a *"placenta annularis composita."* Each of the

eight discoid placentae is pressed against the margins of the two contiguous ones so that the whole set forms a ring or zone encircling the vesicle at right angles to the long axis of the uterus.

The most detailed account of the armadillo placenta yet published is that of Fernandez, who describes several important early stages of this structure in connection with his account of the early development of the Mulita.

Chapman ('01), gives a detailed description of the after-birth of a single specimen of Dasypus sexcinctus. Excellent figures of all structures involved accompany the text. As seen from the foetal side the placenta appears to be truly discoidal in form, but on the maternal side the distribution of the villi is decidedly different from that usually found on that type of placenta. The markedly arborescent villi are arranged in a broad, somewhat lobose ring around the margin of the disc, leaving the centre of the latter free of villi, a condition strongly reminding one of a much earlier stage in the development of the placenta of Tatu novemcinctum, when the original saucer-shaped Träger has begun to produce villi along the free overgrowing margin, but has a comparatively non-villous central area. The forked connection of the umbilicus with the placenta is almost identical with that found in our species. In view of these striking similarities in the placental details of the two species one is led to conjecture that the conditions found in six-banded armadillo closely approximate the ancestral conditions of the more highly specialized armadillos, of which Tatu hybridum seems to be the most pronounced example and T. novemcinctum the next.

In view of the fact that there has yet appeared no complete and consecutive account of the history of the placenta of any species of armadillo it seems worth while to devote a special chapter to a description of the conditions seen in our species.

For the earliest condition it will be necessary once more to call attention to the youngest embryonic vesicle of Fernandez. Here we find surrounding the true embryonic layers the trophoblast, which is attached to the uterine mucosa by means of a thickened disc or plug of trophoblast tissue, called the Träger. This attachment disc is to be considered as the primary placenta. As the

vesicle develops the Träger assumes a saucer-shaped form, as seen in vesicles 10 and 18 (figs. 12 and 14).

It will have been noted that, owing to the inversion of germ layers, the whole yolk-sac region of the vesicle is covered externally with entoderm, and that the trophoblast layer of this region, which in species with a diffuse placenta ultimately forms the outer lining of the villi, has practically disappeared. In the Träger region, however, the original trophoblastic epithelium persists in a somewhat modified form. This region of the vesicle consists of an inner layer of mesoderm, at this time rather thin and free of blood vessels, and an outer trophoblastic layer of true epithelial character, from the surface of which protrude branching and anastomosing cords of trophoblast tissue, which give to the Träger a characteristic rough or ridged appearance (fig. 12). These cords of cells appear to function at first as adhesive pads in that they no doubt serve to give the vesicle a firmer grip upon the uterine wall.

In the primitive streak stage these Träger cords, when examined histologically, show themselves to be composed of solid masses of cells with large nuclei and deeply staining cytoplasm, surrounded by a rather flattened layer of epithelium continuous with that covering the general surface of the Träger. Mitotic figures are of frequent occurrence among the cord cells, showing rapid cell proliferation. In some respects the appearance of the tissue suggest a glandular function, and it may well be that from it a secretion is given off which subsequently facilitates the penetration of the villi into the uterine mucosa. That these cords of cells are of trophoblastic origin seems certain, for the mesoderm, the only other layer in this region of the vesicle, is a thin membrane entirely separate from the trophoblast, which at this period it has not begun to invade. The Träger cords then must be formed by a process of rapid local cell proliferation which causes masses to protrude from the surface and frequently to overgrow it to such an extent that they appear to be almost completely constricted off (fig. 9).

Taking the primitive streak stage as the last phase of the primitive placentation, we may note that the Träger occupies roughly

one-third of the area of the embryonic vesicle (the remainder consisting of the yolk-sac region), that the embryos are attached to the Träger by paired bands of mesoderm, equivalent to the belly-stalk of the primates, and that the central area of the Träger is freer from thickenings than the periphery.

The function of the Träger or primary placenta appears to be not so much nutritive as merely adhesive, since there are at this time no blood-vessels in it by means of which nutriment might be conducted to the embryos. It is highly probable that whatever nutriment reaches the embryos comes to them by a process of osmosis through the thin wall of the yolk-sac region of the vesicle.

The formation of the secondary placenta occurs entirely within the confines of the Träger and involves at tne beginning practically its whole area. A very instructive stage in the development of the placenta is seen in vesicle 18, (figs. 14 and 15). Here the Träger epithelium has been pushed out into short scaly villi, which show a tendency to overlap one another as well as the margin of the yolk sac region. These protuberances have been invaded by a stroma-like mesenchyme, which has arisen from the original thin mesodermal epithelium lining both Träger and yolk-sac regions of the vesicle. The free ends of the scale-like villi are tipped with masses of solid gland-like tissue derived by the breaking up of the branching cords of earlier stages into numerous knots which are carried out to the extremities of the individual villi. Although the general Träger epithelium which surrounds the villi has persisted in the form of a rather thick syncytial layer the knots are bare of covering except for the presence of an extremely thin layer of much flattened and scattered cells. The knot cells therefore are in a position to come into most intimate contact with the uterine tissues and probably serve as organs of penetration, softening the maternal tissues by means of a secretion and forcing open a path for the villi, in much the same way as the diamond tips of drills cut away the harder materials and open up a path for the shaft. These Träger knots forming the tips of the villi appear to persist throughout almost the entire foetal life in a form practically identical with that just described.

The tip of one of the branches of an arborescent villus is shown in fig. 11. The terminal knot of cells is seen to be practically naked, while farther down in the villus are shown blood vessels containing nucleated blood cells.

Although the formation of villi occurs at first over almost the entire area of the Träger, somewhat more advanced stages clearly show the beginning of a tendency for them to become restricted into four distinct patches near the boundary line between the Träger and yolk-sac and around the umbilicus of each embryo. The villi of other regions cease to grow and remain short, as in fig. 3, even flattening down into small rounded prominences which probably serve no nutritive function. Small patches of these flattened villi are scattered over the central area of the Träger as well as between the newly formed placental discs of the various embryos.

During this period the Träger area of the vesicle has been growing more rapidly than the yolk-sac region, the boundary between the two remaining at all times definitely marked. In fig. 3 is shown semidiagrammatically the conditions in vesicle 11 in which four discoid placentae are clearly marked off from the surrounding areas of scattering flat villi. At this stage the placentation is obviously discoid for each embryo.

In vesicle 14, (fig. 4) a decided change is in evidence. The four formerly quite separate discs have undergone a considerable increase in diameter and have come into very intimate contact along contiguous margins. This fusion is more complete between the placentae of embryos I and II and between III and IV than between II and III or I and IV. The significance of this is discussed later. A further change is seen in that the villous margin of the Träger region has overgrown the yolk-sac region (not fusing at this time with the latter) and has extended the placental area of the vesicle along the sides of the cervix cavity as far as the os uteri. Judging by the size and abundance of the arborescent villi in this placental annex it seems obvious that it plays the principal nutritive rôle at this period. One might compare this overgrowing fringe of branching villi to the cricoid placenta of Dasypus sexcinctus.
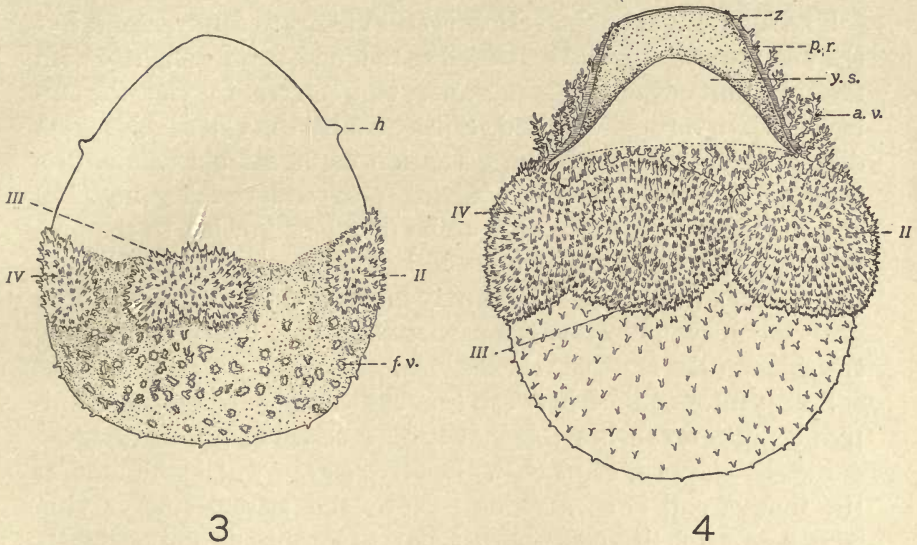
3   4

FIG. 3.   A semi-diagrammatic representation of a vesicle seen from the dorsal side.   II, III, and IV are the placental discs of the embryos so numbered.   Note that those belonging to the paired embryos III and IV are closer together than II and III.   *f.v.*, flattened villi of the Träger; *h.*, horn of the yolk sac.   × 2.

FIG. 4.   A semi-diagrammatic drawing of the dorsal view of a vesicle slightly older than that seen in fig. 3.   This shows the fusion of the placental discs I, II, III, and IV into a zone.   Note that the fusion between the discs of III and IV (of paired embryos) is more intimate than between II and III.   In the cervix region of the vesicle the dorsal part of the overgrowing placental ring, *p.r.*, has been removed to show the smooth yolk-sac lying within (*y.s.*).   The ring was fused with the wall of the cervix at "*z*".   The dotted line lying just above the discs represents the line along which the upper part of the ring was cut.   × 2.

The yolk-sac region of the vesicle is from this period on cut off from all contact with the uterine wall except at the mouth of the uterus where a small circular area remains uncovered by any outer layer.   This condition persists until birth except that the overgrowing ring of arborescent villi undergoes a gradual degeneration, as the placental discs increase in functional prominence until the long, branched villi become mere flattened prominences, which serve only to slightly roughen the membraneous area at the cervix end of the vesicle.

The fundus end of the vesicle is still villous to some extent, but the villi are so small and scattered as to interfere only slightly with the transparency of the membrane. One can readily view the embryos in situ through this end. Subsequently the villi of this region disappear entirely with the exception of occasional small tufts that might readily be overlooked. In several vesicles (nos. 116 and 117) this region was seen to be four-lobed owing to the presence of two thickened bands of tissue crossing each other at right angles (figs. 37). These may indicate a demarkation of the several embryonic primordia earlier than that seen in the differentiation of the embryos themselves.

Stages intermediate between that shown in fig. 4 and the definitive condition can best be shown by a series of photographs.

Fig. 34 shows a somewhat older vesicle, in which the area at the fundus end is seen to be smooth and almost free of villi. The lobing of the composite zonary placenta is only slightly marked.

In fig. 35 is shown the cervix end of a stage slightly more advanced than the preceding one. The heavy coating of arborescent villi is seen to cover the entire cervix end of the vesicle with the exception of the small area that lies across the mouth of the uterus.

The dorsal surface of another vesicle, approximately of the same age as the last, is seen in fig. 36. The vesicle is attached to the shrunken cervix of the uterus. Here is evidenced the tendency on the part of the composite zonary placenta to divide into two double lateral discs. The deep notch occurs between the placental areas of embryos II and III. The small lobe (d. b.) is destined to persist as a bridge between the two lateral discs.

Two farther steps in the development of the definitive placenta are seen in figs. 38 and 39. The vesicle has grown to be several times the size of that shown in fig. 34. Coincident with this great increase in surface the villi in the composite zone have increased in functional importance while those that previously overgrew the yolk-sac region of the vesicle have degenerated, leaving a membraneous area at the cervix pole, which in time becomes as large or even larger than that at the other end of the vesicle.

In fig. 40 is seen a condition slightly more advanced than that described in detail in our preliminary paper. There is now at each pole of the oval vesicle a star-shaped clear area, with a broad, deeply notched placental zone between, which still shows distinct signs of its origin from four discoid placentae. The notches are more deeply cut along the dorsal and ventral lines than along the lateral, where the placentae of the paired embryos I and II and likewise III and IV are so intimately fused as barely to show the points of union.

Shortly after the condition just described the placenta takes on what appears to be approximately the definitive condition. The tendency to form two well defined lateral discs is carried still farther, but in no case have we observed the complete separation of the two placental areas. As a rule the bridge between the two main discs is narrower on the dorsal side than on the ventral, but its narrowness is compensated for by the presence of a heavier coating of villi and by that of rather large placental blood-vessels which serve to connect one main disc with the other. It seems to be almost invariably the case that the division into the two double lateral discs strikes only approximately along the boundary lines of the original discoid areas, for colored injections forced into the placental vessels of individual foetuses run across the narrow placental bridges and invade more or less extensive and clearly marked villous areas of the other main disc. Such a condition is well shown in fig. 41.

The umbilical cords which may be from 18 to 20 centimeters long are attached rather near the fundus margin of the placentae except in rare cases where five foetuses occur and involve the crowding of one or more unbilical cords away from the margin.

Although a litter of young armadillos was born in the laboratory we were not fortunate enough to secure the after-birth and therefore cannot describe this final stage of the placenta. A comparison of the size and degree of development of the new-born young with the oldest foetuses in our possession convinces us that the conditions just described stand as definitive. Yet Lane in his reconstruction of the after-birth of the single individual under observation fails to find connecting bridges between the main discs. He

may have observed a rare case in which the line of separation into lateral discs passes exactly between the placental areas of the two dorsal and the two ventral embryos. Moreover we find no such clearly marked non-villous areas at the two poles as he describes. The smooth area at the cervix end is in all of our specimens very small and circular in outline, while that at the fundus end is only vaguely outlined and frequently shows patches of flat villi.

Any attempt to classify a placenta with the above history meets with grave difficulties, as one might conjecture from the multiplicity of terms applied to it by different writers. Kölliker in his original description of the conditions of the embryonic membranes of T. novemcinctum refers to the placenta as discoidal and deciduate. Milne-Edwards considers it to be compound zonary in structure. Beddard describes it as dome-shaped and deciduate; while Lane suggests the term "zono-discoidalis indistincta," subdividing Strahl's class "zono-discoidalis" into two varieties, "distincta and "indistincta."

Somewhat similar placental conditions, as found in T. hybridum, are designated by von Jhering as indications of a "placenta annularis composita." Chapman's use of the term "deciduate cricoid" appears to be apt for the placenta of the six-banded armadillo.

Of all these terms the one that appeals most strongly as descriptive of a certain rather persistent phase in the development of this multiformed structure is that used by von Ihering, "placenta annularis composita," but one must not forget that at first it is simply discoidal, then cricoid, then tetra-discoidal, later annularis composita, and finally incompletely doubly discoidal.

If animals are to be classified according to the form of their placentae, a method of classification that is fortunately falling into disrepute, it would be very difficult to classify the nine-banded armadillo, unless we arbitrarily decide to select some particular developmental phase of the placenta as a criterion for classification. In such cases one would be led to chose either the primary or the definitive condition and would thus call the placenta either "simply discoidal" or "incompletely doubly discoidal." Other terms scarcely find a rational basis.

The conjecture that the compound placenta of T. novemcinctum has been derived without any fusion of four embryonic vesicles from a condition similar to that described by Chapman for Dasypus sexcinctus, is very tempting in view of the evident close relationship of the two species and the striking resemblance that exists between them in the details of the placenta, umbilicus and other structures. This if true would furnish one of the most cogent proofs of polyembryony, since we find in the more highly specialized species a quadruple placenta, which at a rather early period closely resembles the definitive placenta of a more primitive species that gives birth to single young or to twins.[3]

VI. History of the Amnion

From Fernandez's description of his earliest stage it is clear that the common amniotic cavity is at first the hollow of the ectodermic vesicle, which, through the inversion of germ layers, has come to lie within an envelope of entoderm. Regional differentiation of this ectodermic vesicle produces the ectodermal portions of the embryonic primordia, which are at first contained within a single vesicular amniotic cavity. Subsequently the individual embryos sink into pockets in the floor of the common amnion, which has evidently become fused to the walls of the yolk-sac at the cervix pole of the embryonic vesicle. The posterior end of each embryo has become fixed by means of the primordium of the belly-stalk to the margin of the Träger, and consequently, as the yolk sac gradually increases in size, the embryos are drawn away from the common amnion, retaining connection with it only by means of slender tubes, the amniotic connecting canals (figs. 12 and 14). It has been shown that each pair of embryos withdraws from the

[3] We are informed by Mr. Robert D. Carson, superintendent of the Philadelphia Zoölogical Garden, that a female six-banded armadillo in captivity gave birth to:
1. A single male, on May 10, 1901.
2. Twin males, on April 6, 1902.
3. Twins (male and female), on July 19, 1902.

common amnion into a single pocket and leaves for a short distance a single connecting canal. Later each member of these pairs loses its connection with its partner and acquires its own canal. This secondary separation of the pairs produces a forking of each of the original two connecting canals, a condition that persists for a long time.

After the embryos have left the common amnion the latter probably becomes functionless and ceases to grow. Fortunately however it persists with all of its connections through a considerable developmental period, furnishing evidences of polyembryony and of embryonic pairing. In fig. 44 it is shown still typical in form with its connecting canals entire but with their lumens interrupted with plugs of tissue. The regions between the plugs have become distended through local secretion of amniotic fluid, so that the canals as a whole present a decidedly moniliform appearance. In fig. 45 a somewhat more advanced stage of degeneration in these structures is seen. The common amnion can no longer be recognized but the canals are still clearly defined. Each of these shows a number of pronounced bead-like swellings, one of which may represent the remains of the common amnion. These canals may persist until stages as advanced as that shown in fig. 33, but are seldom to be detected in later stages.

The posterior amniotic processes, which in early stages were seen to be closely associated with the development of the allantois, do not persist in so marked a form in our species as in the Mulita. Only in rare cases does one see any traces of these structures at a period later than the five to seven somite condition (fig. 15). In vesicle 17, however, one of the embryonic amnia is connected by means of an amniotic canal with a sac as large or larger than the common amnion but lying at the opposite pole of the vesicle. This condition is no doubt exceptional and may be accounted for on the supposition that the posterior amniotic process of one of the embryos, on account of its unusual length, protruded far down into the Träger region, came into contact with and united with it, and subsequently swelled into an amniotic sac at the point where its terminal bulb fused with the Träger wall.

Another exceptional condition is that seen in fig. 46, where branching from a typical amniotic canal of one of the embryos, is an accessory canal running to an empty amniotic sac at the center of the Träger. Such a condition is doubtless due, as was stated in another place, to the presence of the remains of a degenerated fifth embryo. Teratological amniotic structures similar to those just described were observed in a number of other cases. In most instances there seems to be no doubt that they represent the retarded or degenerate remains of supernumerary embryos. The frequent occurrence of similar rudimentary embryos in Tatu hybridum and in our own species seems in itself a strong piece of internal evidence of specific polyembryony, for, on the basis of the origin of the several embryos from separate eggs, it would be difficult to understand why some should develop into complete embryos, and others, in the same vesicle and under practically identical conditions, should meet with so little success.

After the closure of the lumens of the various amniotic canals all communication between the four or more amnia is cut off; and henceforth each embryo has its own separate amnion in as true a sense as in those mammals that produce several entirely independent young. The developmental history of these envelopes is moreover in no important way different from that of other mammals except that in late stages a gradual fusion occurs, first of all with the wall of the chorionic vesicle and later with one another, where, through the pressure of growth their walls have come into contact.

Various representative stages in the later history of the amnion are seen in the photographs herewith presented. In fig. 44 the amnia may be seen to lie rather closely applied to the bodies of the embryos. In fig. 33 the cavities of the individual amnia have increased greatly in size and the sacs have assumed an ovoid form with the narrower end directed toward the cervix pole of the vesicle. In fig. 34, an external view of the fundus end of a somewhat older vesicle, the amnia are seen pressed against the membraneous area of the Träger, producing at points of contact an added transparency, reminding one of windows through which the embryos can clearly be viewed.

Even after the embryos have reached a length of 4 cm. the amniotic sacs are still quite free from one another, but a little later they begin to fuse along contiguous surfaces. Not until about a month before birth however do they become inseparably bound together. After the fusion is complete the amnia occupy the entire cavity of the vesicle and divide it into (normally) four quadrants of equal size, each running from pole to pole. This nearly definitive condition was described in detail in our preliminary account and needs no further attention here. In fig. 46 the edges of the amniotic partitions separating adjacent embryos may be seen at "*a*." The umbilical cords are always attached just to the left of the partitions.

VII.   HISTORY OF THE ALLANTOIS AND THE UMBILICUS

The early history of the allantois was shown to be very intimately bound up with that of the belly-stalk or primitive umbilicus This intimate connection persists as long as the allantois retains a distinguishable structure. In stages of the degree of advancement shown in vesicle 17 and 11 (figs. 1 and 44) the entodermal allantois is seen as a slender cord of cells more or less closely fused with the umbilicus and showing here and there traces of a former lumen. The outlines of the mesodermal allantois, however, are no longer distinguishable from the tissues of the belly-stalk. The allantois of the armadillos seems then to be entirely vestigeal in later stages of development.

The umbilicus arises directly from the primitive belly-stalk, which was shown in the description of vesicles 10 and 18 to consist of paired flat bands of mesoderm uniting the posterior end of the embryo to the margin of the Träger or primitive placenta. That the mesodermal allantois contributes some tissue to the definitive umbilicus has already been intimated, but at no time do allantoic blood vessels function. The placental circulation is carried on exclusively by the umbilical vessels, paired arteries and veins. Each artery arises along the inner margin of a belly-stalk band, while each vein forms in the scroll-like outer margin. In later stages the two bands fuse at a short distance from the vesicle

and continue to the body of the embryo as a single somewhat flattened cord. The forked connection between the cord and the vesicle is maintained as a characteristic feature of the placentation. In the definitive condition the umbilicus measures from 18 to 20 cm. in length and about 1 cm. in greatest diameter. The veins are longer than the arteries and take an open spiral course along the flattened edges of the cord.

VIII. Pairing of the Embryos

In our preliminary paper attention was called, in treating of the nearly complete identity of the four embryos, to indications of a still closer resemblance between the individuals of the right and left hand pairs. In attempting to derive the four embryos from the blastomeres of the four-cell stage the following suggestion was offered: "This possible interpretation receives a striking confirmation in the fact that the four embryos can be arranged into two pairs, the individuals of which approach almost complete identity; and these identicals are not only adjacent to each other but are also attached to placental discs that are closely united. If all four embryos are derived from a single egg, this is exactly what we should expect to find; for surely the individuals derived from one of the blastomeres of the two-cell stage ought to be more nearly similar to each other than to the individuals of the other blastomere."

The subsequent acquisition of a large amount of additional data has served only to strengthen our conviction concerning this strong tendency toward pairing among the four embryos: a tendency that expresses itself in the method of separation of the embryos from the common amnion; in the fusion of the four discoid placental areas into two double lateral discs; in the different rates of development seen in the embryos of a single vesicle; and in the closer resemblance, as a rule, between the paired embryos of one double placental disc than between the embryos in general.

The forked arrangement of the amniotic canals, as was pointed out in connection with vesicles 10 and 18, shows that the embryos retreat from the common amnion in pairs and that only when at

some distance from the latter do the individuals of a pair sever their intimate connection and acquire separate amnia. Subsequently these embryos show their pairing in their mode of attachment to the definitive placental discs, embryos I and II being attached to the right hand disc and III and IV to the left.

Fernandez calls attention in the case of the Mulita to the exact identity in stage of development among the embryos of a set. That this is not always the case in our species is well brought out by a comparison of figs. 30 and 31, two embryos from vesicle 18.

Fig. 30 represents embryo III, and IV was identical with it. Fig. 31 was taken from embryo II but would serve equally well as a figure of I. The difference in degree of development between the two pairs is well marked not only in the number of somites (5 in III and IV and 7 in I and II), but in the conditions in the head region and in other parts.

It is not likely that a difference in rate of development between the two pairs is of common occurrence, but the clear case of it just presented seems worth recording not only on account of its rarity but because it serves to emphasize the tendency of the individuals of a pair to be alike, but somewhat different from the equally identical opposite pair.

Although of very common occurrence the pairing of embryos on the basis of resemblances in the total number of scutes in the nine bands of armor, is not without exception. In many cases the pairing is so marked as to be startling, as for example in one case where I and II each has 555 plates and III and IV each has 548; or in another case where I and II have respectively 551 and 552 and III and IV have respectively 560 and 559. In many other cases the pairing is obvious but not so clean cut.

There are on the other hand two cases where there was a close resemblance between three embryos, but one was strikingly different, as for example where II, III, IV have respectively 544, 545, 543 and I has 549; or again where I, II, III have respectively 562, 565, 564 and IV has 573. Finally two cases occurred in which, if any pairing at all exists it appears to be between I and III and between II and IV, as for example where I and III have respectively 544 and 546 while II and IV have 550 and 548.

On the whole however, in spite of these exceptions, the general rule holds good, that the closest resemblances occurs between paired embryos.

In this connection it should be mentioned that even where there is exact resemblance between the individuals of a pair in the total number of scutes in the nine bands of armor, there is no perfect correspondence with respect to individual rows. The resemblance in total numbers of scutes is however, a matter of more importance than the exact manner of their arrangement into rows, which is a secondary process. Each primary scute is the equivalent of a well defined hair group and these groups, as can be seen in other regions of the body, are quite definite units, although subject to more or less shifting before reaching their final arrangement into rows. In a subsequent paper we expect to make a special study of variation and heredity in the elements of the armor and shall in this place refrain from any more detailed reference to the subject.

Another source of data, however, which furnishes striking evidence of pairing is seen in connection with a fairly common tendency for regional fusion of adjacent bands of armor, or for the occurrence of interrupted and of incomplete bands in definite regions. Such atypical conditions occur in from three to four per cent of all cases, a fact that we have established from an examination of considerably over a thousand shells. This comparative rarity of occurrence, while it renders the collection of data on pairing and identity difficult, gives to such data an added value, in that chance resemblances are very unlikely to occur.

Only four cases of strikingly atypical armor arrangements have so far been discovered in the collection of foetuses now in our possession. In one case in embryos I and II there occurred a remarkably atypical scute arrangement in the first band of armor, while III and IV were quite normal. In a second case I and II showed a slight fusion between the first two rows at the right hand margin, while III and IV showed a much more extensive fusion in exactly the same region. The pairing in this case was only a matter of degree of fusion, but there was a decided difference in extent of the region of fusion in the two pairs. In a third

case III and IV exhibit almost precisely the same atypical condition, a short interruption in the first band a little to the left of the median line; II has an interruption in the same band, involving considerably more than half of the total length of the band, while I, although appearing to be perfectly normal, seems to have carried the tendency toward the suppression of a band to the extreme in that the whole band is lacking. In a fourth case one of the four embryos shows a short fusion between the first two rows on the left hand side, while the other three are perfectly normal.

Three out of four cases, then, furnish strong evidence of pairing, while the fourth case, which is after all atypical only to a minimum extent, affords an exception, whose weight can scarcely be sufficient to discredit the evidence of the other cases.

Although the pairing of embryos is not always perfectly obvious the cumulative evidence in favor of its general occurrence is convincing and must have some fundamental significance, an understanding of which is undoubtedly closely bound up with the early developmental mechanics as we shall attempt to show.

It has occurred to us that the division of the four-scalloped placental band into right and left lateral discs might be dependent upon the fact that the blood supply of the uterus comes from the paired ovarian blood vessels that enter the uterus laterally. It is true that the paired embryos, with very few exceptions are located on the same side of the uterus, but that the pairing is in any way causally related to the fact of their location near the entrance of a single maternal blood vessel is highly improbable, because the maternal blood does not reach the embryos.

It has also been suggested that the close resemblance between the individuals of a pair might be due to admixture of foetal blood, but we have demonstrated by the use of colored injections that the placental area of each embryo is sharply circumscribed and that no blood passes from one embryo to another. A common blood environment then cannot be held accountable for the near approach to identity seen in the pairs. Moreover it has been shown that long before there was any sign of the definitive placentation, and hence before there was any circulation of blood, pairing of embryos was evident in the relationship of the amniotic

connecting canals and in one case, in the degree of development of the embryos.

These observations force us to the conviction that the orientation of the vesicle in the uterus and the pairing of the embryos are expressions of the cleavage polarity and symmetry of the ovum. The cell products of the first two blastomeres would occupy the right and left halves of the early blastocyst and the daughter cells derived from the first two blastomeres would normally hold their relative positions as quadrants of such a blastocyst, so that, although it may not be possible to note any definite demarkation of embryonic primordia until a much later stage, they may be well defined from the first. When however pairing seems to exist between diagonally opposed embryos it might conceivably be due to a shifting of blastomeres in the four-cell stage, which could readily occur in such loose cell aggregates as prevail in early mammalian cleavage stages. A shifting upwards of two diagonally placed blastomeres and a consequent shifting downward of the other two would bring about a recombination of blastomeres into two new pairs without interfering with the hereditary tendencies of the individual units. Such an appeal to the imagination of the reader would scarcely be justified were it not the logical outcome of a failure to explain the conditions on any other basis. We are much inclined, in spite of Fernandez' failure to note any indication of a demarkation of separate embryonic areas in his earliest vesicles, to believe that such areas exist from the beginning and express themselves as separate primordia only on the differentiation of embryos. This view is in direct opposition to that of Fernandez who holds that up to the time when the separate embryos are distinguishable, the vesicle is a single embryo.

IX. CONDITIONS IN VESICLES CONTAINING FIVE FOETUSES

Out of a total of seventy embryonic vesicles there occurred three in which there were five foetuses. In all of these the sex could be determined and, curiously enough, they were all males. Whether or not this condition is universal could not be determined. If however it should prove that all five-embryo sets are males it

would mean that sex is determined by certain conditions in the egg. With only three cases in hand a discussion of the matter would be unprofitable.

In two cases out of three it was possible to enumerate the scutes in the nine bands of armor and on that basis to determine the varying degrees of resemblance among the embryos.

The occurrence of five embryos involves a decided asymmetry of the placental and amniotic elements and an atypical arrangement of the embryos. In each case the condition of two main lateral discs was maintained, but one of these discs, the one to which three embryos were attached, was considerably larger than the other. An examination of the larger disc shows that in each case it is composed of only two, not three, primary discs. One of the primary discs, on the side where three embryos are attached, is twice the normal size and to it are attached in symmetrical fashion the umbilical cords of two embryos. Apparently there is no regularity about the position of the double disc. In one case the double disc is ventral, and in the other two right lateral in position. Believing that the two embryos attached to a single primary disc are the equivalent of one typical embryo, we shall give them the same number, as for example, I and I'.

The following conditions are found in vesicle 91, the relative positions of the embryos being indicated in the diagram of the placenta, represented as cut open along the narrow dorsal bridge and laid out flat (fig. 5). The number of scutes in the nine bands of armor are indicated on the figure. It will be noted that there is distinct pairing on the normal side of the vesicle, between embryos III and IV; that the resemblance between the two embryos on the large disc (I and I') is equally close; but that there is a wide difference bewteen these two embryos and the single embryo on the same side (no. II).

In vesicle 108 somewhat similar conditions exist, but the vesicle is laid open along the ventral bridge (fig. 6). Embryos II and II', having a common primary placental disc, are identical in the number of scutes but widely different from embryo I, which is attached to the other primary disc on the same side of the vesicle. Embryos III and IV are quite different from those on the other side, but are fairly similar to each other.
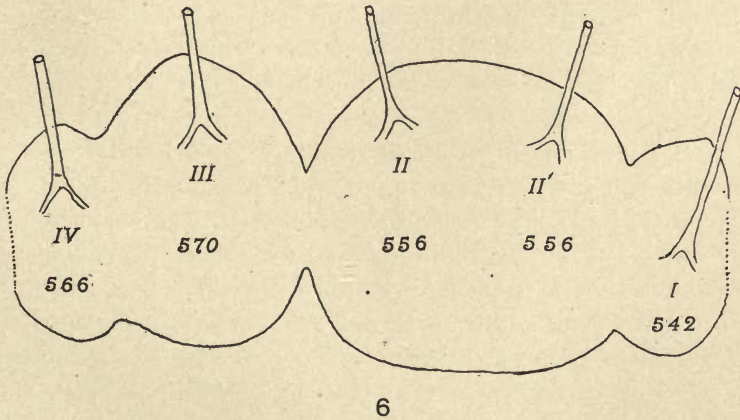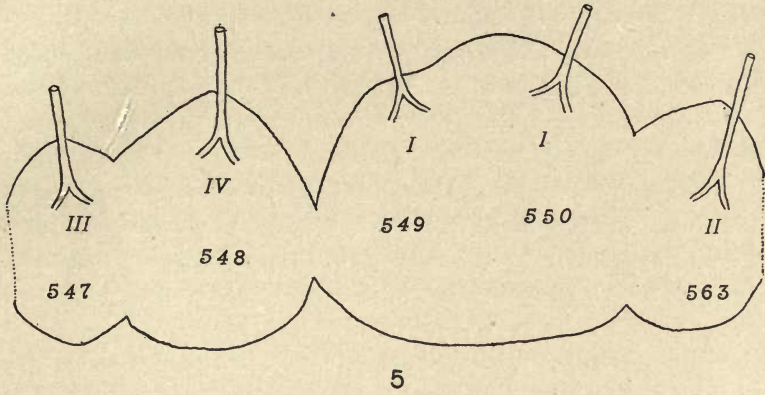
FIG. 5.   Diagram of the placenta of vesicle no. 91, showing the placentation and the numbers of scutes in the nine bands of each embryo.   Cut open along the dorsal notch.

FIG. 6.   The same scheme for vesicle 108.   Cut open along ventral notch.

In the case of the third five-embryo vesicle a satisfactory enumeration of the scutes was not found possible, but the position of he large disc was the same as in 108.

In all three cases the amnia of the three embryos occurring on the same side are irregularly arranged. Instead of occupying whole quadrants of the subspherical vesicle the amnion of one or more embryos is forced away from one end and crowded past the opposite end, thus causing the amniotic partitions to run diagonally across the placental discs instead of taking a meridional course from pole to pole as in typical cases. The relative positions of the embryos is of course correspondingly irregular so that one is immediately struck by it when the vesicle is first exposed to view.

The high degree of mal-adjustment seen in these vesicles would seem to indicate that the occurrence of more than four embryos is the expression of a coenogenetic tendency to carry polyembryony a step farther by a doubling of the present typical number of embryos. In the Mulita this condition has been attained and there exists a strong tendency to double again, as seen in the frequency of vesicles containing nine or more embryos. It appears probable to us in view of the occurrence of one case of twins in our collection, that in T. novemcinctum specific polyembryony had its origin in the acquisition of a habit of producing identical twins in a fashion similar to that seen in other mammals, that the inversion of germ layers made it easy for this tendency to express itself still more fully in the habitual production of four embryos. The production of more than four embryos in our species seems to involve so great a disturbance of a very accurate adjustment of embryos and embryonic membranes that it seems highly improbable that a larger number of embryos will ever become typical.

It would be interesting to find out whether there is in T. hybridum any tedency of the embryos to arrange themselves into two groups corresponding to the right and left sides of the vesicle. A study of Fernandez' photographs (figs. 1 and 2) would seem to indicate that such is the case. It is hoped that this matter will receive some attention and that the degree of resemblances among the embryos of the various sets will be determined.

X. The Question of Identity of Embryos

In the case of identical or monochorial twins the question of close resemblance has been much discussed and the impression seems to prevail that the individuals of a pair show such marked similarity in their finer details of structure as to be practically identical.

In our earlier contribution to this subject we were inclined to look for the resemblances between the embryos of a litter and to understimate the value of the points of difference. Now however that we feel that the question of specific polyembryony has been established, the differences among embryos interest us more than the resemblances, because they indicate a rather marked degree of versatility in the hereditary possibilities of a single fertilized germ cell.

The only point of unfailing identity among the individuals of a litter is that of sex. In 38 cases where the sex was definitely determined there was no exception to the rule that all embryos in a vesicle are of the same sex.

So far as dimensional differences go there is again practical identity, although in a few cases there seemed to be a slight difference in the size of the two pairs. In comparing one individual with another we were forced to admit that they differed only in the minutest details, such as the number of scutes in the armor. A comparison on this basis is just about as searching as would be a comparison of the number of feathers in a given feather tract of two birds, or of the hairs in a given hair area of two mammals. We have for the present limited our comparison to the total number of large scutes (with corresponding underlying bony plates), in the nine moveable bands of armor. The extreme range of variability in the total number of these plates (in all of the individuals so far examined) is rather wide, running from 511 to 620, a range of 109. In a number of cases the individuals of a litter exhibit a range of only five or six scutes, but as a rule the range is wider, averaging in all cases studied twelve, or less than one-ninth of the total range of our sample of the species. Whether or not this represents a closer esemblance than exists between

the individuals in a litter of rats or other mammals cannot at present be determined.

Although the difference between the two pairs of a litter may on the average be rather marked, that between the individuals of a pair seldom exceeds three scutes and averages in all cases observed less than three, while cases of absolute identity in the total number of scutes is of frequent occurrence.

It will be remembered also that in our discussion of pairing a considerable mass of evidence was adduced to show that even in atypical scute arrangements a high degree of identity existed between pairs, while in most cases the pairs differed greatly from each other. All of these observations go to show that the identity between the individuals of a pair is a very real thing but that the there is nothing approaching true identity between the pairs. The condition may well be described as a case of double identical twins.

XI. Specific Polyembryony and the Determination of Sex

The first clue to the existence of polyembryony in the armadillos was furnished by the discovery that all of the individuals of a litter are of the same sex. This together with his observation of a common chorion, led von Jhering to surmise that all of the embryos of a vesicle arise from a single fertilized egg. That this flash of insight foreshadowed the discovery of a truth has been sufficiently demonstrated, we believe, by Fernandez for Tatu hybridum and by us for T. novemcinctum.

Identity of sex then is in some way closely bound up with the phenomenon of polyembryony. Presumably all of the individuals of a litter are of the same sex because they have been derived from a single fertilized ovum; but this presumption involves the corollary that sex is determined in the germ before any demarkation of embryonic rudiments has occurred. The only alternative is that similarity of environmental conditions during the developmental period has the effect of producing offspring all of the same sex, an alternative with no factual basis, as is shown by the

following observations: that at a very early period each embryo is surrounded by its own amnion; that a little later each draws maternal nutriment from a separate area of the uterine wall; and that there is no admixture of foetal blood. We are therefore driven to the conclusion that sex is determined before there occurs any splitting of the single germ into separate embryonic primordia.

Opinions differ as to the exact period at which this splitting takes place. Fernandez maintains, on the basis of his studies of the early blastocyst of the mulita, that there is no trace of poly-embryony until after the two primary germ layers have been laid down. What he probably means is that previous to this time there is no visible demarcation of the germ layers into isolated blastodermic areas. That the real separation of embryonic rudiments occurs at a much earlier period, even during the early cleavage stages (in our species at the four-cell stage), seems probable in view of the discovery of pairing among the embryos, a phenomenon for which no other explanation offers itself; and by the observations of Marchal, ('04), and Silvestri ('06), on the parasitic hymenoptera, where each embryo in a set takes its rise from a single cell of a rather advanced cleavage stage.

It seems highly probable then that the tissues involved in each of the four quadrants of an embryonic vesicle, whether or not they may show a demarcation, do really arise as the lineal descendants of one of the first four blastomeres. In this sense the four embryos are delimited at the four cell stage. It is hardly to be expected that any demarcation would be visible before the beginning of the period when the separate embryonic shields are differentiated.

The question as to the exact period of separation of the several embryonic rudiments is one that cannot at present be definitely settled. Even if one should be fortunate enough to obtain the early cleavage stages it is improbable that he would be able to observe any essential departure from the usual plan of mammalian cleavage, for a blastomere of the four cell stage would have the same appearance whether it were destined to produce a whole or only a quarter of an embryo.

It seems probable from our studies of the ovaries that the tendency to polyembryony is inherent in the unfertilized egg, which is the seat of a developmental vigor somewhat more intense than that exhibited in the ova of other mammals. This extra expresses itself sometimes by parthenogenetic divisions and at other times in the formation of fairly regular morulae within the confines of the Graafian follicles. That polyembryony is simply a more normal expression of the same superabundant energy in the female germ cells seems highly probable, and we would offer this as a tentative explanation of the physiology of polyembryony, pending an exhaustive study of a large collection of ovaries.

Taking it for granted then that sex is determined in the undivided oosperm, the question naturally arises as to which of the two germinal elements is the sex determiner. Cytological examination of the ovaries reveals no dimorphism of the ova. They all have 32 chromosomes and are equally alike in other respects. The possibility that sex might depend on which of the two ovaries produced the egg that became fertilized as suggested by the work of Dawson ('09). This writer maintains on observational grounds that in the human being the male producing ova come from the right and the female producing ova from the left ovary. The corpus luteum served to indicate which ovary functioned in any given pregnancy. In the armadillo we have an exceptional opportunity to put Dawson's theory to a test, for the corpus luteum of this species is a very prominent feature of the ovary that has functioned. A study of our data reveals the fact that the corpus luteum is found with almost equal frequency in right and left ovaries, which coincides with the exact equality of male and female litters. Unfortunately for the theory, however, there is no correlation between the sex of the embryos and the dextrality or sinistrality of the functional ovary. Out of twenty cases in which the right ovary contained the corpus luteum, the sex of the embryos was male in seven and female in thirteen; while out of thirteen cases in which the left ovary held the corpus luteum, the sex was male eight times and female five. Evidently then the position of the functional ovary has no determining influence on sex.

There is on the other hand excellent evidence that the male cell may act as a sex determiner. Studies of the spermatogenesis of our species show that the spermatogonial number of chromosomes is in all probability 31, one less than the oögonial. There is moreover in the reduction division a very definite and obvious odd chromosome, which precedes the other chromosomes to the pole of the spindle and serves to institute a dimorphism of the spermatids. That the odd chromosome is concerned with the determination of sex is as probable for the armadillo as for the insects and other forms in which it has been described. Both rest on the same observations. Since it is our intention to make a detailed study of the cytology of the germ cells in this species, it must suffice for the present to have indicated the sort of external evidence of polyembryony and of sex determination we have at our command.

The discovery of so clear a case of an accessory chromosome in a mammal is in itself worthy of mention, since it brings us a few steps nearer to the discovery of the physiology of sex determination in man. In addition to the intrinsic value of this discovery, however, we are afforded another strong proof of specific polyembryony, in that it is highly improbable, on the basis of the origin of the embryos of a vesicle from several fertilized eggs that each of these eggs would be fertilized by the same kind of spermatozoon. Such a possibility could be realized only through the instrumentality of selective fertilization, the occurrence of of which has never been successfully demonstrated.

XII. Summary of Evidence for Specific Polyembryony

1. The uterus is simple, resembling that of the primates, which give birth typically to one offspring at a time.

2. There is never more than one large corpus luteum in the ovaries of a pregnant female.

3. In over 90 per cent of vesicles the number of normal embryos is four, a number that suggests their origin from the blastomeres of the four-cell stage. It is also unlikely that this number of ova would so often be given off at the same time.

4. The fact that all of the embryos of a set are invariably of the same sex strongly suggests their origin from a single fertilized egg.

5. The definite orientation of the embryos in the vesicle, and of the vesicle in the uterus, precludes the possibility of their origin from several eggs, even though these might conceivably be simultaneously given off from the ovary.

6. The inversion of germ layers presents a condition in both Tatu hybridum and in T. novemcinctum, which could not be attained by the union of several eggs to form a single vesicle. This is the strongest piece of evidence for specific polyembryony that has been advanced, and, to our minds, is practically conclusive.

7. The Träger or primitive placenta, common to all four embryos, is the morphological equivalent of that seen in the monembryonic vesicles of certain rodents.

8. The overgrowing fringe of arborescent villi seen in middle stages of gestation reminds one strongly of the cricoid placenta seen in the monembryonic vesicle of the six-banded armadillo, figured by Chapman.

9. The existence of partial or rudimentary embryos is evidence against the idea that the several embryos have been derived from separate eggs, for it is difficult to understand why some should develop perfectly, while others, under the same environmental conditions, should have so little success.

10. The pairing of embryos points to the origin of each pair from one of the first two blastomeres.

11. The presence of an accessory chromosome in the male germ cells suggests that the spermatozoon is the sex determiner. On this basis the fertilization of several eggs always by the same kind of spermatozoa seems highly improbable.

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REFERENCE LETTERS

a.a., amniotic attachment to wall of vesicle.
al., allantois.
al.en., allantoic entoderm.
al.ms., allantoic mesoderm.
am., amnion.
am.c., amniotic cavity.
am.c.c., amniotic connecting canal.
a.v., aborescent villi.
b.b., belly-stalk bands.
b.c., blood cords.
b.s., belly-stalk.
b.v., blood vessel.
c., cervix of uterus.
c.a., clear area of Träger.
c.am.c., canal of the common amnion.
c.e., canal enlargement.
c.l., corpus luteum.
co., cœlome.
d.b., dorsal bridge.
d.n., dorsal notch.
ec., entoderm.
en., entoderm.
e.v., extra chorionic vesicle.
ex.c., extra embryonic body cavity.
f.g., fore-gut.
f.n.t., floor of the neural tube.
f.t., Fallopian tube.
f.u., fundus end of uterus.
f.v., flattened villi of Träger.
h.f., head fold.
h.ms., head mesoderm.
h.p., head process.
i., infundibulum of the Fallopian tube.

i.l., intestinal loop.
l.s., lymph sinus.
ms., mesoderm.
ms.co., mesodermal connection.
m.p., medullary plate.
n.ch., notochord.
n.g., neural groove.
n.l.l., notch of the left lateral lobe.
o., ovary.
p.am., posterior amniotic process.
p.am.c., posterior amniotic cavity.
p.p.h., protochordal plate of Hubrecht.
p.p., primitive pit.
p.r., placental ring.
p.s., primitive streak.
s., somite.
s.am.c.c., supernumerary connecting canal of amnion.
s.t., sinus terminalis.
s.v., scale-like villi.
t.m.p., tip of the medullary plate.
tr., Träger.
tr.c., Träger cavity.
tr.e., Träger epithelium.
tr.k., Träger knots.
u.m., uterine mucosa.
v., villi.
vg., vagina.
y.s., yolk-sac.
y.s.en., yolk-sac entoderm.
y.s.w., yolk-sac wall.
I, II, III, and IV, refer respectively to the ventral, right lateral, dorsal, and left lateral embryos.
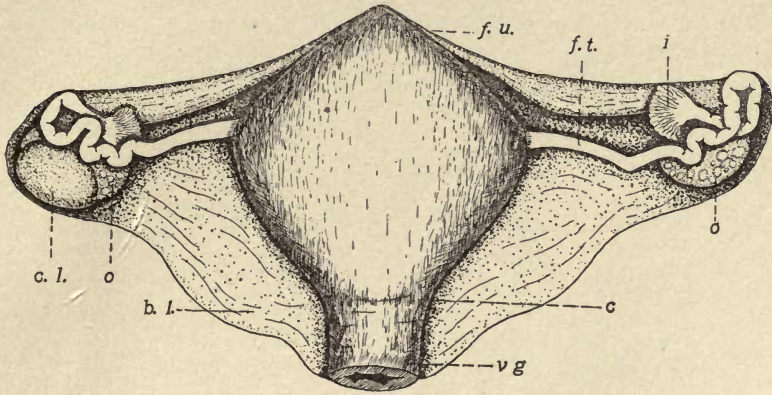
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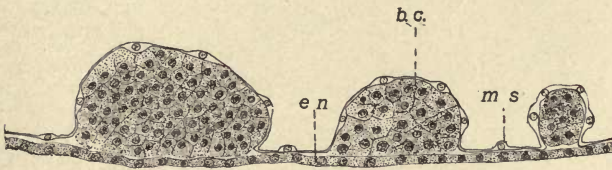
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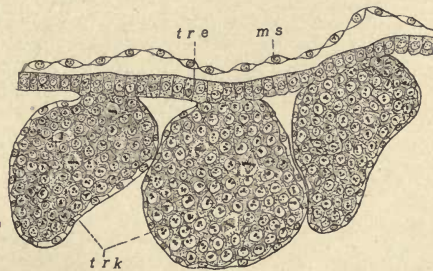
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FIG. 7. The genitalia of an adult virgin female as seen from the dorsal aspect. *b.l.*, broad ligament; *c.*, cervix of uterus; *c.l.*, corpus luteum in left ovary; *f.u.* fundus end of uterus; *f.t.*, fallopian tube; *i.*, infundibulum; *o.*, ovary. × 3.

FIG. 8. Portion of the yolk–sac wall in the region of the area vasculosa (see fig. 15). It shows three blood cords in section. These are made up of a central core of solidly packed cells, *b.c.*, which are surrounded by the mesodermal epithelium, *ms.* × 215.

FIG. 9. Cross section of the Träger of our youngest vesicle (fig. 12), showing three adjacent Träger cords or knots (*tr.k*); *tr.e.*, Träger epithelium. × 265.

FIG. 10.   Cross section of scale-like villi, v., of the vesicle in fig. 14.   The Träger knots are still covered with a thin epithelium.   The epithelium of the villi has become a syncytium.   The mesoderm has proliferated cells which have invaded the villi, but as yet blood formation has not taken place.   × 265.

FIG. 11.   The tip of a villus from a more advanced stage, showing, in addition to the features described in preceding figure, the well developed blood vessels, $b.v.$ × 265.
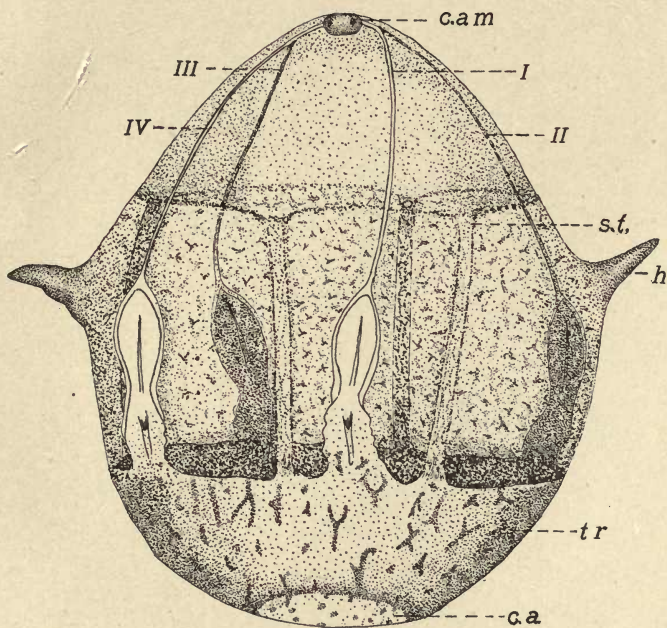
12

Fig. 12. A detailed drawing of vesicle no. 10 as seen from the ventral side as a semi-transparent object. The embryos in white (I and IV) are on the upper side of the vesicle, and since there is an inversion of germ layers, these are seen from their ventral aspects. Embryos II and III are shaded, and lie on the far side of the vesicle. For a fuller description see text. × 9.
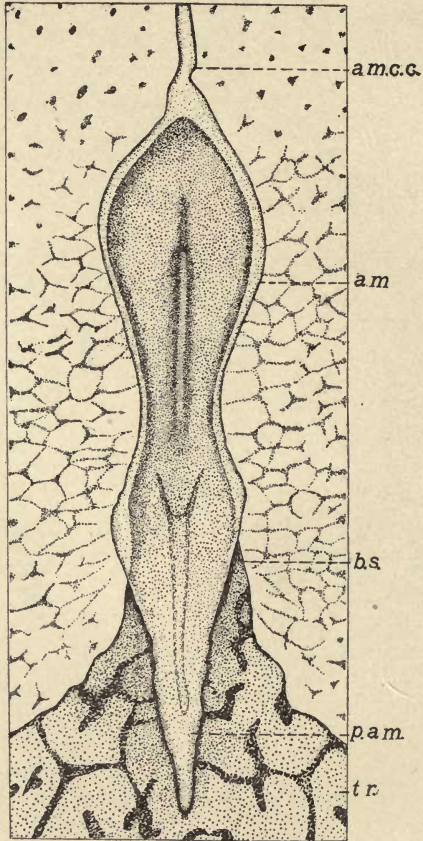
13

Fig. 13.   A detailed drawing of embryo I (fig. 12) as seen from the dorsal aspect, that is, as viewed from the inside of the vesicle.   *am.*, amnion; *a.mc.c.*, connecting canals of the amnion; *p.am.*, posterior amniotic process; *b.s.*, belly-stalk; tr., Träger.   × 25.
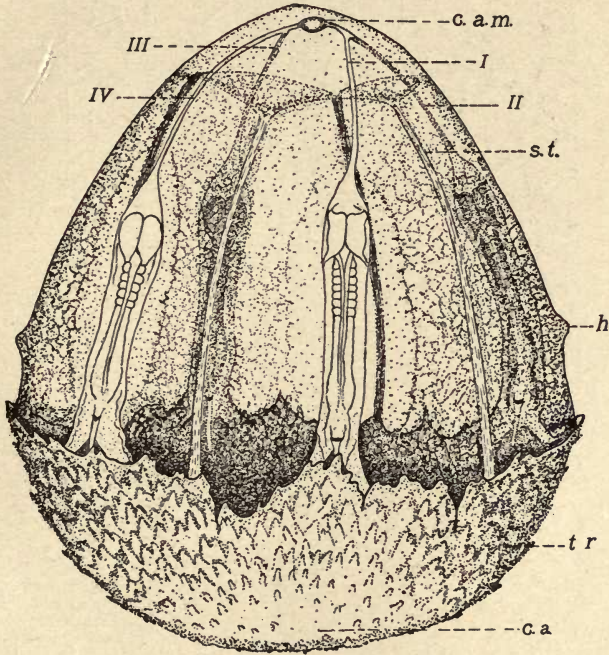
14

Fig. 14.   Ventral view of vesicle No. 18, seen as a semitransparent object.   The Träger is covered with scale like villi, which overlap the lower margin of the yolk-sac.   This vesicle should be compared with that shown in fig. 12, in which the lettering is the same.   × 5.
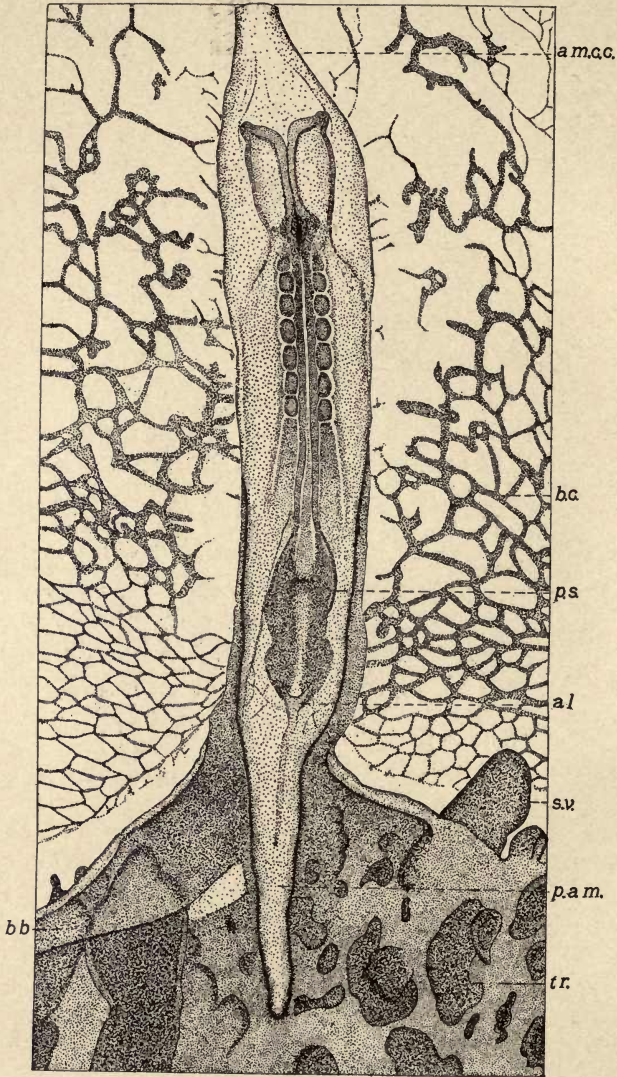
15

Fig. 15.   A detailed drawing of embryo I (fig. 14) as seen from the dorsal side.   A photograph of this embryo is shown in fig. 31.   *al.*, allantois; *am. c.c.*, connecting canal of amnion; *b.b.*, belly-stalk band, note that the band is much more distinct on the left side than on the right; *p.s.*, primitive streak; *s.v.*, scale-like villi; *tr.*, Träger region, which shows the villi as seen from their under sides.   For a fuller description see text.   × 21.

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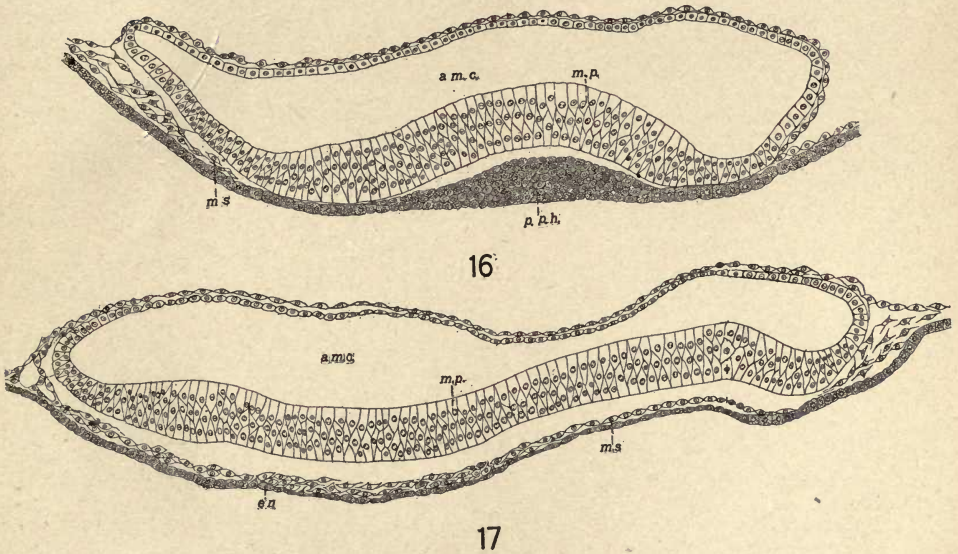
NOTE—Figs. 16–23 represent a series of transverse sections taken through various regions of an embryo from the same vesicle as that shown in fig. 13.

FIG. 16.   A section taken through the anterior end of the medullary plate.   The most important feature of this section is the thickening of the entoderm to form the "protochordal plate" of Hubrecht, *p.p.h.*   × 130.

FIG. 17.   A section taken through the medullary plates at a point lying half way between the fore end of the head process and the anterior tip of the embryo. The entoderm is distinct from the mesoderm, which is scarcely more than one cell thick. × 130
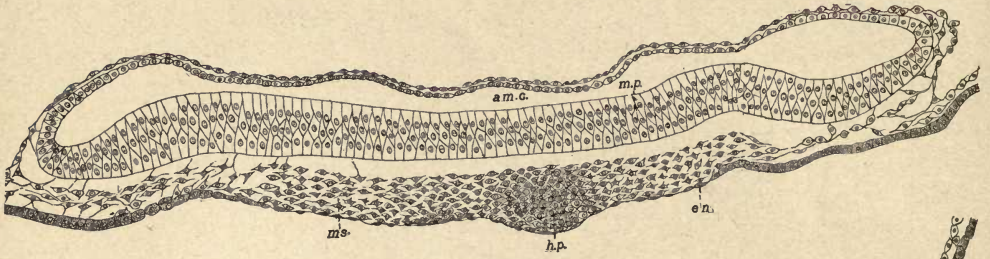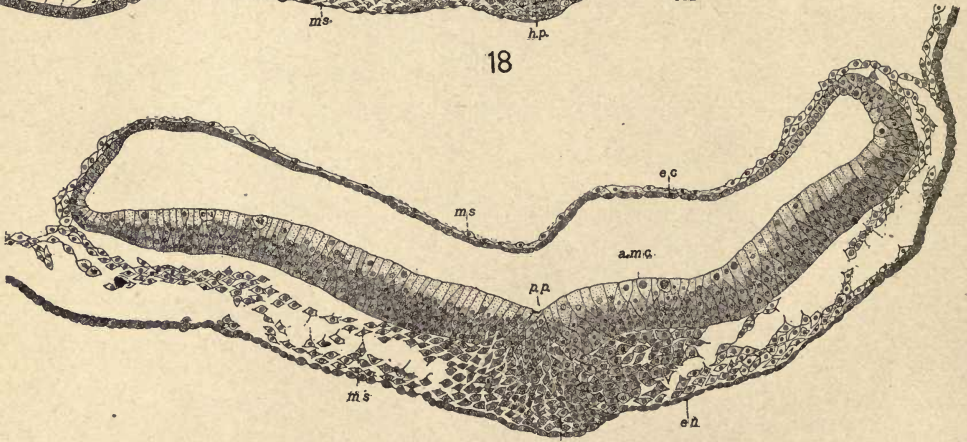
FIG. 18.   A section taken through the middle of the head process.   In this region the entoderm is very intimately associated with the mesoderm, especially in the central part of the section.   × 130.

FIG. 19.   A section taken through the primitive pit.   It shows the primitive streak proliferating mesoderm in the characteristic manner.   × 130.
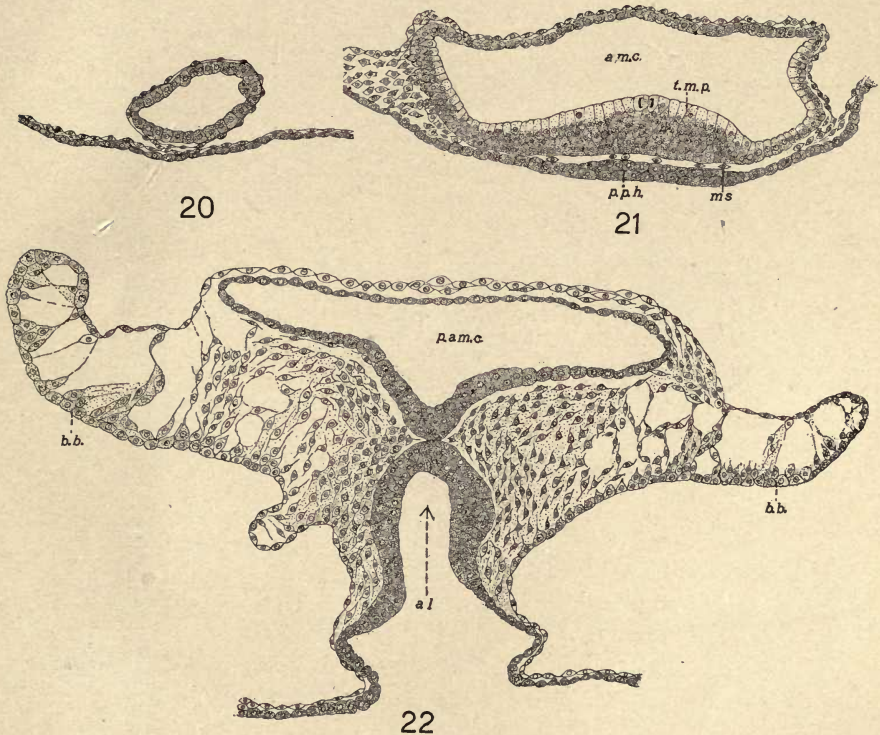
FIG. 20.   A section taken through the connecting canal, which is seen to be composed of two layers, ectoderm on the inside and mesoderm on the outside, and is loosely connected with the mesoderm of the yolk-sac wall. × 143.

FIG. 21.   A section taken through the tip of the medullary plate.   This is the first section that shows the anterior end of the protochordal plate of Hubrecht. Note that there are a few scattering mesoderm cells (*ms.*) that have wandered in between the plate and the ectoderm. × 143.

FIG. 22.   A section taken through the belly-stalk at the level of the mouth of the allantois (*al.*).   The cavity of the posterior amnotic process (*p.am.c.*) does not cover more than one-half the width of the section.   The mesoderm of the belly-stalk extends laterally to form wing-like processes.   These are the belly-stalk bands (*b.b.*) through which the umbilical blood vessels pass to the Träger. × 143.
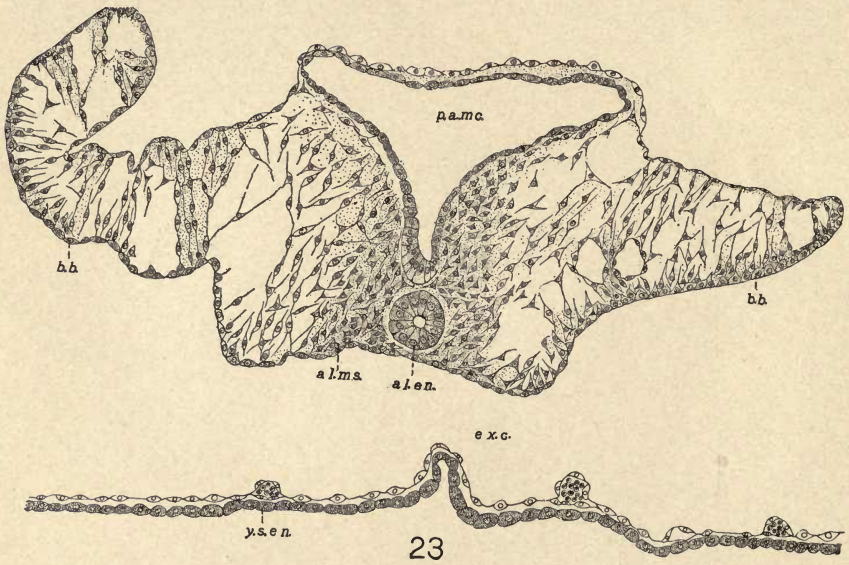
FIG. 23.   A section taken through the belly-stalk near the posterior tip of the allantoic entoderm (*al. en.*).   The mesoderm is indistinctly divided into two portions: (1) that forming the belly-stalk bands, and (2) that part immediately surrounding the allantoic tube—this may be called the allantoic mesoderm (*al.ms.*). The belly stalk is here separated from the wall of the yolk-sac by a space (*ex.c.*), which is only a part of the general extra-embryonic cavity.   × 143.
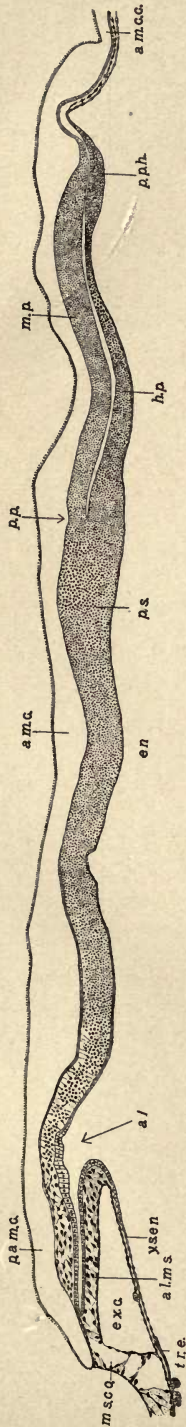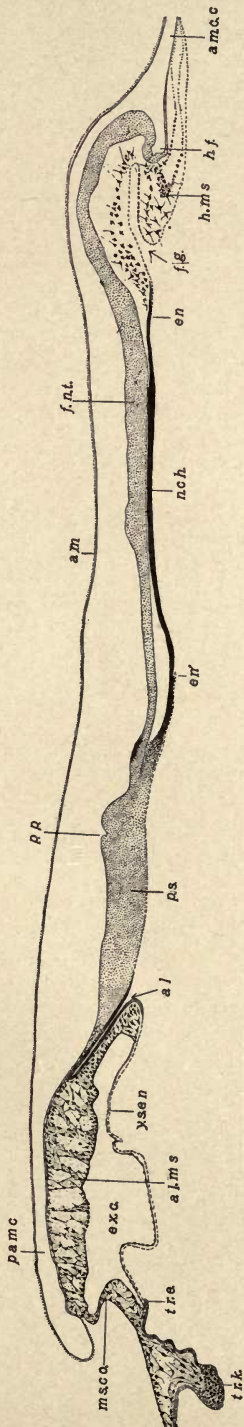
FIG. 24. A semi-diagrammatic longitudinal section of the primitive streak stage. *al.*, mouth of allantois; *ms.co.*, posterior mesodermal connection between the belly-stalk and the Träger; *p.p.h.*, protochordal plate of Hubrecht. See table of reference letters for further explanations. × 47.

FIG. 25. A similar section for the seven somite stage. As compared with the preceding stage, the following are the principal changes that have taken place: (1) formation of the fore-gut, *f.g.*; (2) differentiation of the head mesoderm, *h.ms.*; (3) formation of the notochord, *n.ch.*; (4) reduction in the length of the allantoic entoderm, *al.*; and (5) formation of the head-fold, *h.f.* Attention should be called to the condition of the entoderm. It is wanting directly beneath the notochord, except for a short distance at each end. At the posterior end of the notochord the entoderm turns back on itself for a short distance (*en.'*). × 38

PLATE I

NOTE, figs. 26–29 represent a series of transverse sections of a five-somite embryo.

26. A section through the region of the head-fold. The brain vesicle is in the process of formation, and the neural groove (*n.g.*) has become very deep. The notochord (*n.ch.*) is represented by a row of cells, and to each side of it the entoderm is bayed to form the pharyngeal pouches (*ph.g.*). × 68.

27. A section through the somite region. The somite shows a distinct cavity, and the coelomic cavity is forming. The entoderm is beginning to close in beneath the notochord. × 68.

28. A section through the proximal part of the allantoic tube. The bands of the belly-stalk have become much folded, and contain a number of umbilical blood vessels. The posterior amniotic process has become reduced to a very small tube. × 68.

29. A section through the posterior mesodermal connection (*ms.co.*) of the belly-stalk. The Träger shows the villi in the process of formation. × 30.

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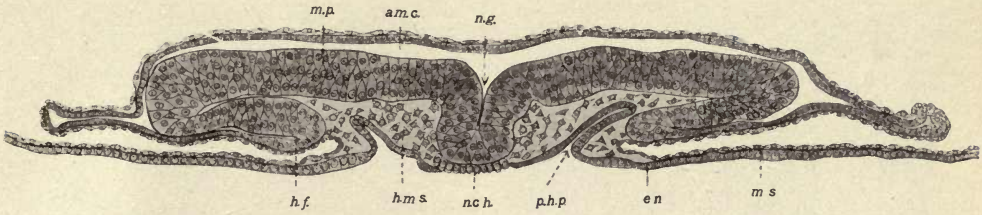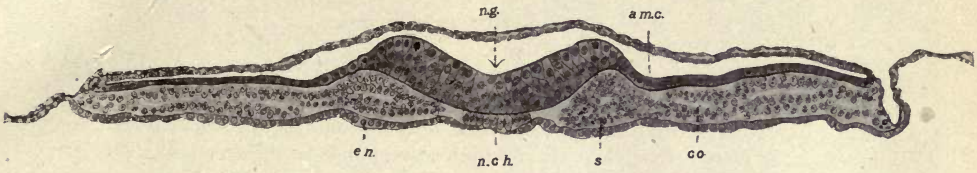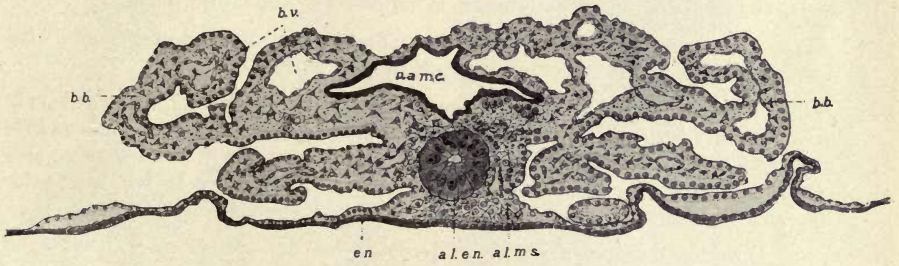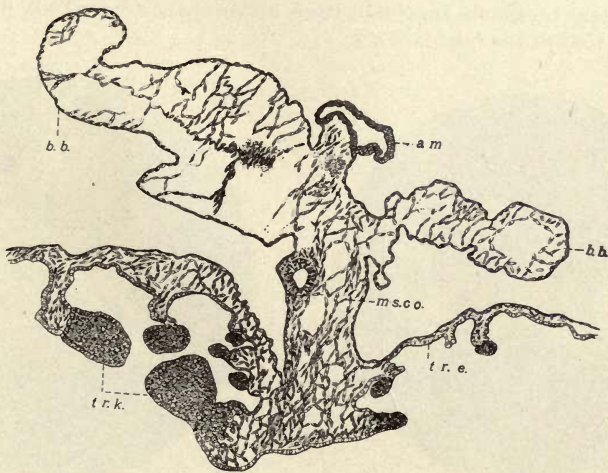
PLATE II

30. One of the five somite embryos (III) of vesicle No. 18 (see figs. 14 and 32). Note how the embryo is attached to the Träger by means of the belly-stalk (*b.s.*). The area vasculosa, like that of the chick, does not extend in to the embryo, but is separated from it by a clear space which corresponds to the area pellucida. On the right is seen the compound sinus terminalis (*s.t.*) lying between the vascular areas of the two contiguous embryos. The posterior prolongation of the amnion is not clearly seen, but its extreme tip is indicated by the leader, *p.am.* × 16.

31. A seven somite embryo (I) of this same vesicle. For a description of this embryo see the detailed drawing shown in fig. 15. × 16.

32. The dorsal view of the vesicle reconstructed in detail in fig. 14. The cervix end is slightly torn and is turned under, consequently the common amnion and its canals are not shown in the photograph. The turning under of the torn piece also makes the vesicle appear shorter than it really is. At *o.m.* may be seen the scale-like villi beginning to overgrow the lower portion of the yolk-sac. × 2.15.

33. A vesicle cut open along the mid-ventral line and spread apart to show the pairing of the embryos. It will be noted that the embryos are arranged so that the right-hand pair (III and IV) is the mirrored image of the left-hand pair (I and II). At this stage the amnia are still distinct, and in shape are oval with the broad end directed toward the fundus. × $\frac{1}{2}$
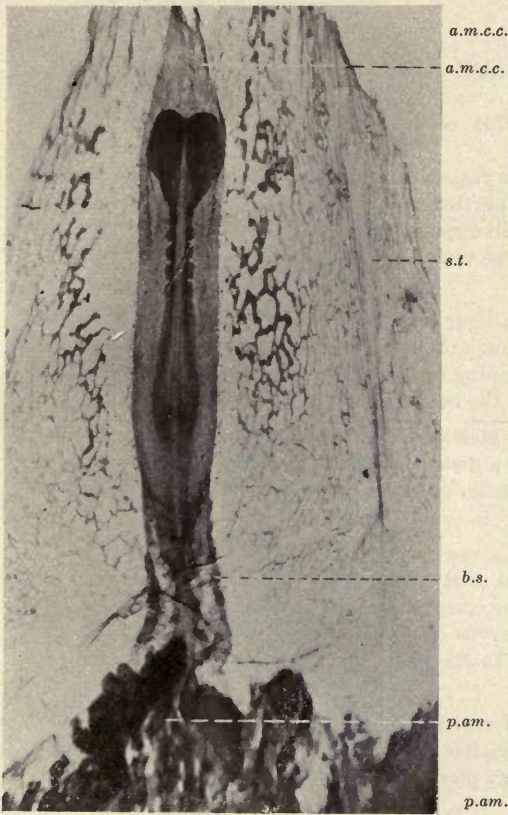
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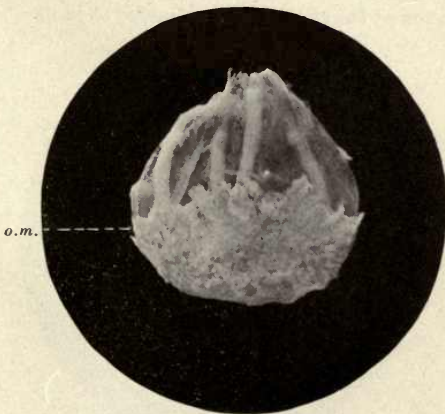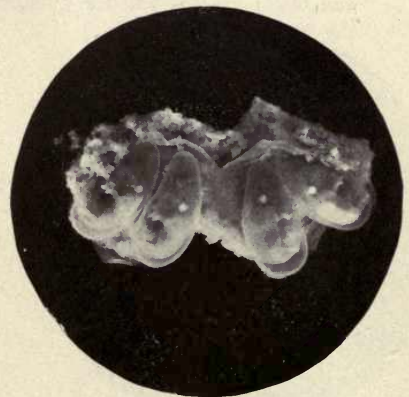
PLATE III

34. A view of the fundus end of a vesicle which contained embryos measuring 31 mm. head rump length.[4] In the portion of the vesicle lying within the margin of the placenta are seen four window-like spots. These are the areas where the amnia come in contact with the wall of the vesicle. The fundus end is now practically free of villi. $\times \frac{1}{2}$

35. A view of the cervix end of a vesicle in which the embryos measured 31 mm. The clear yolk-sac is seen through the opening in the rather thick placental overgrowth. The margin of this opening represents the place where the placenta is attached to the uterine mucosa at the cervix end of the uterus. $\times \frac{2}{3}$

36. The dorsal view of a vesicle which is still attached to the cervix of the contracted uterus. This vesicle shows a distinct placental bridge (*p.b.*) connecting the lateral placentae, and also a number of blood vessels at the fundus end. Embryos 32 mm. in length. $\times \frac{2}{3}$

37. A view of the fundus end of a vesicle which contained embryos measuring 33 mm. This view shows two points worthy of especial note: (1) the four-lobed appearance of the fundus membrane, due to constrictions occurring between the fundus areas of the individual embryos (seen more clearly before fixation); (2) the persistence of a few villi, which in the photograph appear as scattering black specks. $\times \frac{2}{3}$

38. A view of the ventral side of vesicle, with embryos measuring 36 mm. The cervix end of the yolk-sac is clearly visible, and blood vessels are seen at the fundus end. The placental bridge although present is not clearly brought out in the photograph. $\times \frac{2}{3}$

39. A view of the ventral side of a vesicle which contains embryos measuring 53 mm. The division of the zone-like placenta into right and left halves is clearly brought out. The fundus end of the vesicle is now practically free of both villi and blood vessels, and the membranous area at the cervix is much larger than in the preceding figure. $\times \frac{2}{3}$
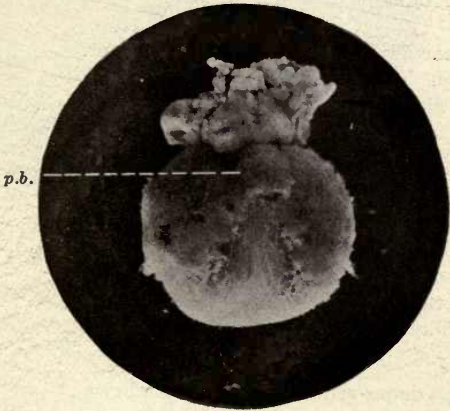
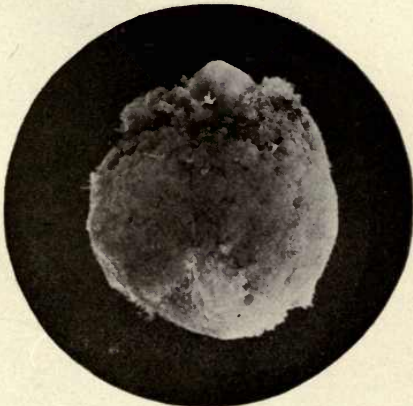[4]Unless otherwise stated, the length of the embryo will mean the head-rump measurement.

DEVELOPMENT OF THE NINE-BANDED ARMADILLO.
H. H. NEWMAN AND J. T. PATTERSON.

PLATE III.

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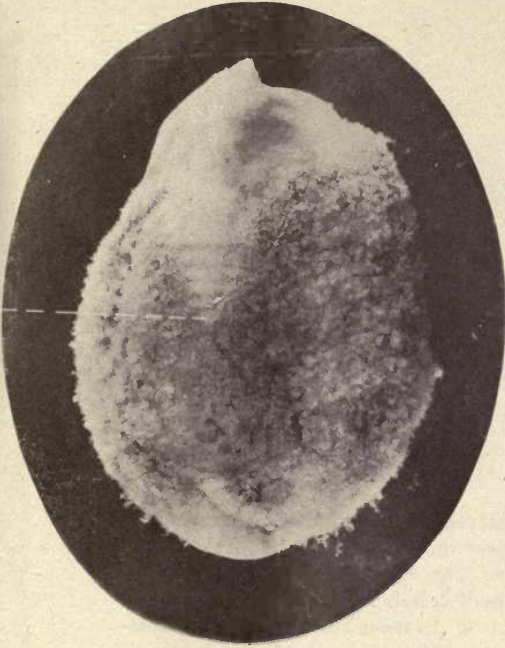
p.b.

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PLATE IV

40. The dorsal view of a vesicle in a rather advanced stage of development. The embryos measure 155 mm. from tip to tip. The dorsal notch, *d.n.*, although extending down to near the meddle of the vesicle, does not completely separate the lateral placental discs. $\times \frac{2}{3}$

41. Dorsal view of a vesicle showing the difinitive condition of the placenta. The placenta is divided into two lateral discs, each of which is distinctly bilobed. The notch between the two lobes of the left lateral (on right) disc is clearly shown in the photograph (*n.l.l.*). The discs are united to each other both on the dorsal and ventral side by placental bridges, the one on the dorsal side (*d.b.*) being the narrower. The original arborescent villi at the cervix end have greatly degenerated, and have become reduced to flat, blunt knobs. The embryos in this vesicle are about 210 mm. from tip to tip. $\times \frac{1}{2}$.

42. Right lateral view of a uterus showing a dorso-ventral bilobing. Embryos are 48 mm. long. $\times \frac{4}{5}$.

43. Ventral view of a pear-shaped uterus, which contained embryos measuring 52 mm. This and the preceding uterus show two of the several forms that have been observed. $\times \frac{1}{2}$
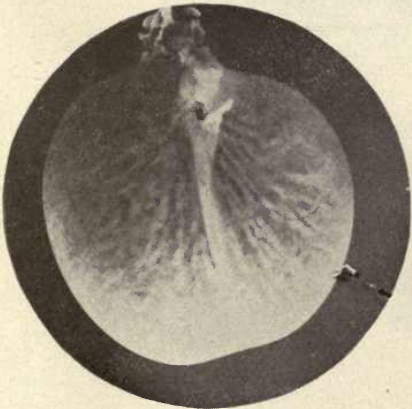
DEVELOPMENT OF THE NINE-BANDED ARMADILLO.
H. H. NEWMAN AND J. T. PATTERSON.

PLATE IV.

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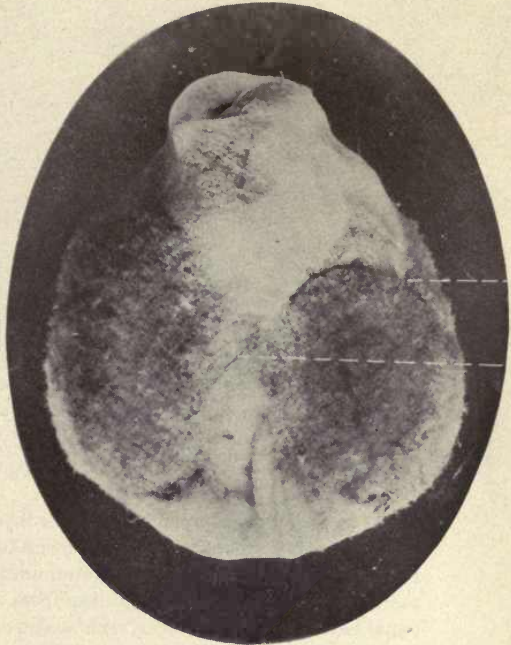
PLATE V

44. A vesicle split open to show the internal relationships of the different parts. The amniotic connecting canals are seen to pass from the anterior ends of the amnia to the spot occupied by the common amnion. This vesicle also shows a supernumerary canal (*s.am.c.c.*) extending from a small vesicle in the Träger wall to the canal belonging to the lower, right-hand embryo. In the entire condition the vesicle measured 24 mm. wide by 29 mm. long. (see fig. 3 for a diagram of the placenta.) Very slightly enlarged.

45. A vesicle laid open in a manner similar to the preceding. At the distal end of each canal is shown a series of bead-like enlargements (*c.e.*). The origin of the canal from the anterior tip of the amnion is shown with especial clearness in the embryo lying nearest the foot of the plate. In the entire condition the vesicle measured 24 mm. wide and 30 mm. long. Very slightly enlarged.
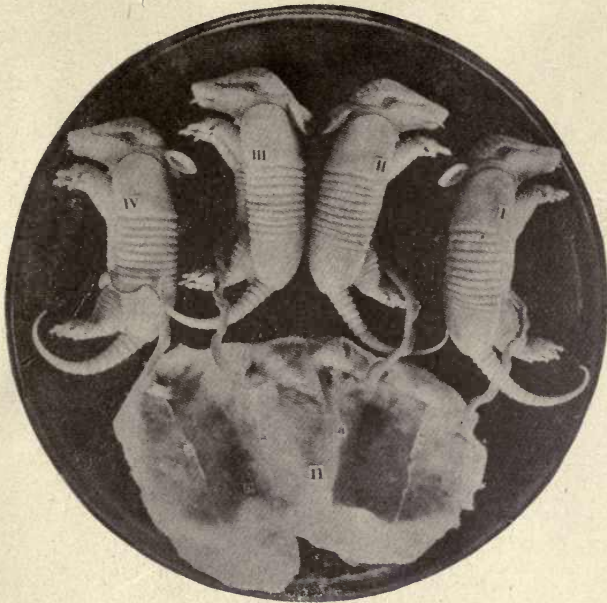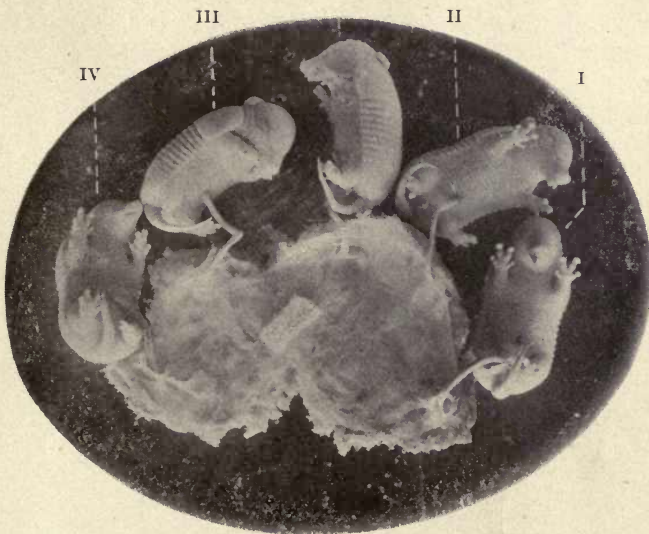
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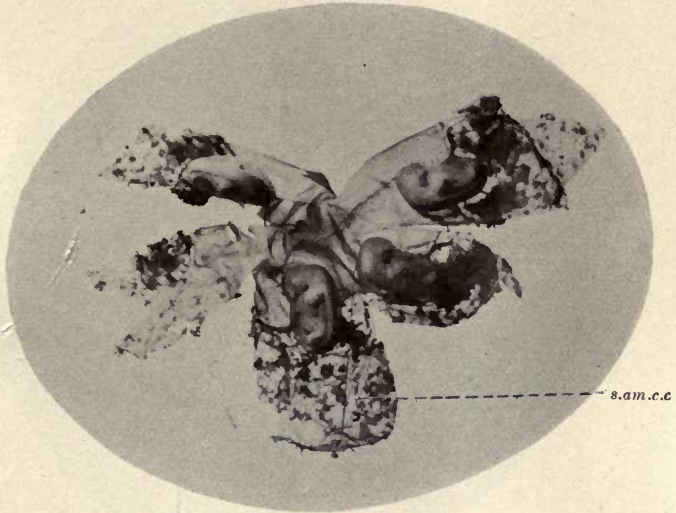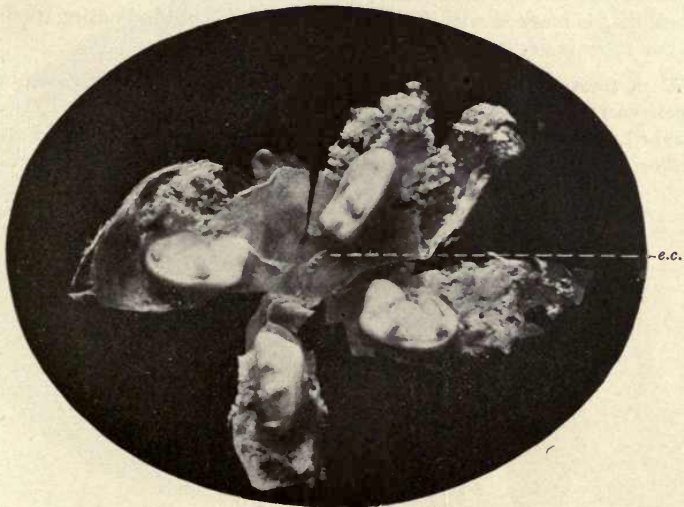
PLATE VI

46. A vesicle cut open along the mid-ventral line to show the relationship of the embryos to each other and to the wall of the vesicle. Each of the four amniotic partitions (a), which have been cut off close to the chorionic wall, lies just to the left of the umbilical cord. These are attached to the wall near the tips of the placental lobes at the fundus end. The left lateral placental disc is indistinctly seen through the chorionic wall, and the notch separating it from the right lateral disc is marked with the larger "n", while that indicating its division into the two lobes is designated by the smaller "n," $\times \frac{1}{3}$.

47. A photograph of vesicle no. 108, which contained five embryos. This vesicle was cut open along the mid-ventral line. Embryos nos. I, II, and II, are attached to the large, right lateral placental disc, and embryos III and IV to the smaller, left lateral. (See text for a fuller description and significance.) $\times \frac{1}{2}$.

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