

# POLYEMBRYONIC DEVELOPMENT IN TATUSIA NOVEMCINCTA.<sup>1</sup>

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THIRTY-FIVE TEXT FIGURES AND ELEVEN PLATES

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## INTRODUCTION

The development of more than one individual from a single egg while not a rare phenomenon among animals, is nevertheless of much biological interest. It has become customary to classify such types of development as asexual or agamic reproduction; but obviously this term has come to include a variety of developmental phenomena, which are exhibited in animals ranging from the Protozoa to the mammals. Among the more common types of agamic reproduction are ordinary binary fission, budding, cyclical parthenogenesis, paedogenesis, and polyembryony.

Upon the basis of certain evidence which has been brought forward from a study of comparatively late embryonic stages, it has been correctly concluded that the type of agamogenesis which has become habitual in the Texas armadillo is that of polyembryony; but so far no one has succeeded in demonstrating the validity of this conclusion. The writer has in his possession a series of young stages which covers the period of early embryonic differentiation, and which represents the material upon which this paper is based. An outline of the more general features of the work has already been given in a preliminary paper (Patterson '12). The facts to be presented in detail are not without a certain interest and significance, not only because they raise to the dignity of an observed fact the claim for polyembryonic development in the armadillo, but also for the reason that they throw a great deal of light upon related phenomena in other mammals. It is unusual to find agamic reproduction in the highest class of animals, and a detailed study of the history of this process is greatly to be desired. Furthermore, there has been considerable speculation as to how 'identical twins' and similar types of development have arisen, and I believe that these studies on the development of the armadillo will at least indicate how these phenomena may have come about.<sup>2</sup>

<sup>2</sup> It is a pleasure to acknowledge here my indebtedness to my friend Mr. F. L. Whitney of the School of Geology for his able assistance in connection with the photographic work. I am also grateful to Mr. F. Pfeiffer, who has greatly facilitated this work by his many successful efforts in obtaining material.

## METHODS

*1. Technique*

For all of the stages described in this paper, I have found Zenker's fluid to be the most useful fixing reagent. Kleinenberg's picrosulphuric acetic acid works well on attached stages, notwithstanding the fact that many embryologists have regarded this fluid as poor for preserving the finer structures. All of the material has been imbedded in paraffin, and the sections cut either 5 or 7 micra thick. Most of the preparations have been stained with acid hematoxylin, although Heidenhain's iron-hematoxylin has been used for some of the earliest stages. Whole mount preparations of the early embryonic vesicles, which were made by the glycerin jelly method, were found to be very useful in the interpretation of certain changes occurring in these young stages.

*2. Method of securing early stages*

In order to understand the methods employed for securing the young vesicles it is necessary to give a brief account of the structure of the uterus. The uterus of this armadillo is of the simplex type, like that of the primates, and gives no evidence, in its external appearance, of being in any way adapted to accomodate a litter of four. Viewed from either the dorsal or ventral sides, the uterus proper resembles a rather blunt spear-head, with the distal or fundus part representing the tip and the proximal or cervix part the shaft end of the instrument. The fallopian tubes, which lie in the plane of the broad ligament, enter the uterus at points lying approximately two-thirds the distance between the cervix and the tip of the fundus.

The external appearance of the non-pregnant uterus varies greatly, both in the virgin and in the old female; but in this connection we are concerned primarily with the condition of the internal surface. In the young female, two-thirds to three-fourths grown, the surface of the mucosa is rather smooth, and gives only faint indications of a folded condition, but in the adult virgin females of two years, and especially in the old females, the uterine mucosa becomes greatly folded. The folds have a general distribu-

tion over the lining of the uterus, except at the tip of the fundus, where there is a four-pointed, cross-shaped area of rather smooth mucosa. The arms of this cross meet each other at approximately right angles, and their common area is the extreme tip of the fundus. This cross also indicates the orientation of the uterus; for two of the arms mark the dividing line between the upper and lower halves of the fundus, and two form a rather broad, shallow groove extending from the mid-dorsal point around to the mid-ventral point of the uterine cavity. These facts are most clearly brought out in an everted uterus (fig. 21).

Each one of the arms forms a distinct furrow leading from the tip of the fundus to the uterine opening of the fallopian tube. As already indicated, the other two arms lead from the center of the fundus to the middle of the upper and lower surfaces, respectively. These usually form shallow furrows which end distally among the folds of the mucosa. For convenience we shall speak of the first pair as the right and left horizontal grooves, and of the second pair as the dorsal and ventral vertical grooves. On account of the fact that the uterine openings are situated slightly nearer the fundus than the cervix end, the horizontal grooves are somewhat shorter than the vertical grooves.

To the student of the early development of the armadillo, the significance and importance of this cross-shaped area can scarcely be over emphasized. The right and left horizontal groove forms the pathway along which the embryonic vesicle passes on its way from the fallopian tube to the tip of the fundus; and the center of the cross, or the central area of the fundus, is the attachment zone or placental area for the vesicle. The discovery of these facts has greatly facilitated the collecting of the early stages. Prior to 1911 attempts were made to obtain the young stages, and various methods were tried, such as that used with great success by English workers in collecting the early stages, namely, that of injecting the uterine cavity full of some killing fluid, and later examining the contents for the embryos. While this method works well where one is dealing with an animal which gives off several eggs at each ovulation, yet in the case of a single egg at each pregnancy the chances of its discovery are few. Furthermore, to any one who

has endeavored to study the fragile mammalian vesicle, the difficulty of preserving the delicate structure even after discovery, is indeed very great.

Early in the fall of 1911, however, the real significance of the cross-shaped grooves, and especially of the horizontal one, was first fully realized, and since then not much difficulty has been experienced in obtaining the free uterine or early attached vesicles. In the living or fresh uterus it can easily be demonstrated that the horizontal groove of each side is virtually a tube, and is therefore, a continuation of the fallopian tube. This is brought about by the depth of furrow and by the depressed condition of the uterus dorso-ventrally.

The best method to follow in seeking to obtain a young vesicle is to cut the uterus along each side in the plane of the broad ligament, beginning at the cervix and extending to a point lying a short distance from the opening of the fallopian tube, and then slowly to evert the fundus portion over the end of the small finger. By this procedure the horizontal furrows are spread wide open, and the vesicle, if present, will be revealed. The tip of the everted uterus is then applied to the killing fluid and the vesicle, if not attached, will float out on the surface of the fluid, and after a few seconds slowly sink to the bottom without distortion. In the case of attached vesicles, the everted uterus is placed in the fluid, and, after fixation and hardening is affected, a properly oriented block containing the vesicle is cut out of the mucosa. The process of obtaining these early free stages may be greatly facilitated by first determining which ovary holds the corpus luteum, and confining one's search to the corresponding horizontal groove.

#### NOTES ON BREEDING HABITS

##### *1. General statement*

During the past five years a considerable amount of information concerning the habits of this animal has been secured by the writer, and it may be worth while eventually to publish these observations; but here I wish merely to make a few remarks on certain phases of the breeding habits.

The breeding season extends over a considerable portion of the months of October and November, and thus any lot of embryos taken at a given time during the period of gestation will present much variation in development. This makes the determination of the length of gravidity difficult and quite uncertain. An exact determination could only be made by breeding animals in captivity. Since a majority of the young are born in the months of March and April, gestation is probably about one-hundred and forty days.

The old females breed first, mating in most cases before October fifteenth, while the second year virgin females continue to breed for some time after this period. Females of one year do not breed except in rare instances. My records for the past five years show just three cases of pregnancy among these young animals, out of at least two-hundred examinations.

In connection with the collecting of material and this incidental study of habits, I have discovered a 'period of quiescence' of the embryonic blastocyst. The fact was first made apparent in 1911, when, after I had started collecting two weeks earlier than in the preceding year, I failed to obtain the cleavage stages, although judging from the condition of development in the vesicles collected in previous years, one would naturally expect to find these early stages during the period of my first collections in 1911.

Again in 1912, I began collecting material two weeks earlier than in 1911, and much to my surprise obtained blastocysts in almost exactly the same condition as those secured during the preceding fall. Practically all of these vesicles lie free within the uterine cavity, either in the horizontal groove or in the region of the attachment zone (placental area).

It is evident from these data that the embryonic vesicle remains for some time lying free within the uterine cavity. Just how long this period lasts, I am unable to state; for practically every old female taken at the earliest date (October 15) at which I have collected, possesses a free blastocyst. How long such blastocysts have been in the uterine cavity it is, of course, impossible to determine; but I should judge not very long, because two vesicles

taken from the fallopian tubes show a development almost as far advanced as that of some vesicles taken from the proximal parts of the horizontal grooves.

Taking all the facts into consideration, I estimate the 'period of quiescence' to last about three weeks; that is from about the middle of October to the third or fourth of November. There are exceptions to this, but not sufficient in numbers to modify the general conclusions. Of the thirty-four free blastocysts obtained in 1911 and 1912, twenty-eight of them were secured within this period.

There is another line of evidence which more or less supports the above conclusion. I refer to the condition of the corpus luteum. In all the females from which the free blastocysts were taken the corpus has attained approximately its maximum size. Unless we attribute a phenomenal rate of growth to the corpus, it is necessary to assume that quite a long period has elapsed since ovulation took place, in order to account for its large size. The short fallopian tube of the armadillo precludes the suggestion that the egg has spent a great while in traversing this passage, so that we must conclude that any arrested development in the egg takes place after it enters the uterine cavity.

So far as the writer is aware, the only other mammal in which a similar quiescent period in the development of the blastocyst occurs is the deer. According to Assheton ('98) Bischoff ('54) states that in this animal the embryo, upon reaching the so-called morula stage, enters upon a 'period of quiescence,' and remains unaltered for some weeks.

It is scarcely correct, however, to state that the blastocyst of the armadillos, during the entire period of quiescence, is in a state of arrested development, because it undergoes certain progressive changes. So far as one can tell from a study of sections, no mitotic divisions occur, but the vesicle increases in size, due to the accumulation of fluid within its cavity, accompanied by the attenuation of the trophoblastic cells. Furthermore, the differentiation of the ectoderm and entoderm from the inner cell-mass is completed while the blastocyst lies free within the uterine cavity.

*2. Available material*

The desirability of having at one's disposal a close series of early stages for a study of this kind is self-evident; but from what has been stated in the foregoing pages it is clear that many obstacles stand in the way of securing such a series. This is particularly true with reference to the cleavage stages. In another season this much desired material can probably be obtained by beginning to collect at a period still earlier than that of the preceding year. During the season of 1912 about a dozen uteri, with attached fallopian tubes and ovaries, taken from females in which signs of recent pregnancy were evident, were preserved. The study of some of these has led to the discovery of early blastocysts lying within the lumen of the fallopian tube. It is therefore highly probable that if a complete series of cleavage stages is to be had, it will be necessary to pursue the laborious method of making sections of the fallopian tubes from females showing signs of recent fertilization.

In this connection I should like to point out a possible source of error, and one that must be carefully guarded against. In sectioning the ovaries of females well started in pregnancy one occasionally finds undivided eggs in that part of the fallopian tube which is situated close to the ovary. Such cases are to be attributed to ovulations that have occurred after normal ovulation and fertilization have taken place. The fact that the nucleus in these eggs may undergo division does not signify anything of unique importance, since it must be regarded simply as an expression of the same tendency to parthenogenetic development which frequently is seen in matured ova still confined within the ovarian tissues of this animal.

In table 1 is given a list of all the free blastocysts which have been secured during the seasons of 1911 and 1912, including the two taken from the fallopian tubes. The first vertical column gives the catalogue number of the specimen, arranged chronologically; the second, the date at which the vesicle was taken; the third, the diameter of the vesicle in millimeters, measured in 70 per cent alcohol; the fourth, the ovary, right *R* or left *L*, from

which the egg came; the fifth, the age of the mother; the sixth, the number of the figure, in case the specimen is illustrated in the paper; the seventh, remarks. Unless otherwise stated, under remarks, the vesicle was taken from the right to left horizontal groove of the uterine cavity. In most instances differences in size indicate differences in the degree of differentiation among the several blastocysts, although there is some variation in vesicles showing corresponding differentiations.

The table brings out several interesting points, and to those that will not be specifically treated in subsequent sections we must direct a few remarks.

In twenty-nine of the thirty-two cases listed the ovary from which the egg came was determined; and the record shows that fourteen were derived from the right ovary and fifteen from the left, thus indicating that there is no tendency for one ovary to function more frequently than the other. In two of the remaining cases I failed to make a record on this point, but in the third (No. 297) both ovaries were enlarged. This would indicate that occasionally the two ovaries function simultaneously, although in this particular case I was not able to find a second egg. There is of course the possibility that the one ovary gave off an egg which failed to become implanted, and that a second ovulation, involving the other ovary, followed immediately.

It was stated above that the 'old' females, that is females which have previously borne young, breed first, and that the second year virgin females come on later. The table brings these facts out clearly. Thus, of the eighteen cases with complete records, which were taken in October, fourteen (Nos. 230, 287, 288, 291, 292, 295, 297, 300, 304, 305, 309, 320, 325, 326) were from old females, while only three were from virgin females, that is females that had never before borne young. Of the eleven cases involving second year females, eight vesicles (Nos. 243, 244, 245, 251, 258, 259, 261, 335) were taken between November first and eighth; while two of the remaining three were secured late in the month of October, both on the 26th (Nos. 311, 315). Finally, of the three vesicles obtained from the first year females, that is females that had been born during the previous winter or spring, two were taken

TABLE I  
*Free embryonic vesicles*

NO. OF SPECIMEN	DATE	SIZE IN MM.	OVARY	AGE OF MOTHER	FIGURES	REMARKS
230	10-30-11	0.319	L	Old		
242	11- 1-11	0.307	R	Old		
243	11- 1-11	0.495	?	2d year		
244	11- 1-11	0.263	L	2d year	9	From left fal. tube
245	11- 3-11	0.324	R	2d year		
249	11- 3-11	0.429	R	Old	14,41	Lying free on placental area
251	11- 3-11	0.420	L	2d year		
253	11- 3-11	0.306	R	Old		
258	11- 8-11	0.384	L	2d year		
259	11- 8-11	?	L	2d year		
261	11- 8-11	?	R	2d year		
268	11-14-11	0.330	L	1st year		
284	12-12-11	?	?	1st year		
287	10-15-12	0.312	L	Old	6	
288	10-15-12	?	L	Old		
291	10-18-12	0.404	R	Old		Lying free on placental area
292	10-18-12	0.480	L	Old		Lying free on placental area
295	10-18-12	0.380	R	Old		
296	10-18-12	0.376	L	2d year	10,37	From left fal. tube
297	10-19-12	?	R=L	Old		
300	10-19-12	0.656	L	Old	12,42	Lying free on placental area
304	10-22-12	?	R	Old		
305	10-22-12	0.348	L	Old		
309	10-26-12	0.432	L	Old		
310	10-26-12	0.360	R	?	7,36	
311	10-26-12	0.504	L	2d year	11,47	Lying free on placental area
315	10-26-12	0.380	R	2d year		
318	10-26-12	0.336	R	1st year		
320	10-28-12	0.387	L	Old	13,39,40	
325	10-28-12	0.290	R	Old		
326	10-28-12	0.339	R	Old		
335	11- 2-12	0.220	R	2d year	8,38	

very late in the breeding season, one (No. 268) on November 14, and the other (No. 284) on December 12.

In table 2 are listed all the early attached or implanted vesicles which are directly referred to in the subsequent parts of the paper. They are arranged, so far as possible, in the order of their degree of development. Vesicle No. 311 of the preceding table should probably be included in this list, and No. 300 should almost certainly be included. They were both lying free upon the placental area when first observed, and for that reason are included in the list of unattached specimens; but inasmuch as the early attached vesicles are so easily dislodged, it might well be that these two vesicles were loosened during the process of everting the uterus. Their size and structure most certainly point to this conclusion.

In determining the size of those vesicles which had gained a firm attachment to the mucosa it was found best to measure the base of the vesicle, as this gave the most trustworthy results. In everting the uterus, however, the mucosa of the placental area may become slightly stretched or otherwise distorted, thus spreading the base of the vesicle, and consequently giving a measurement that is abnormally large. This is the case in No. 256. Furthermore, all of the vesicles listed beyond No. 332 were measured from the microscopic preparations, and consequently after the shrinking effects of dehydration, clearing, and imbedding had come in. The sizes indicated for these vesicles are too small as compared with those listed above No. 332 (cf. 233 and 307).

#### DEVELOPMENT OF THE BLASTOCYST

##### *1. The monodermic blastocyst*

The transformation from the so-called morula stage to the hollow sphere, or 'monodermic blastocyst,' has not been observed in the armadillo. The youngest normal stage which I have at my disposal already shows the finished product of this transformation. However, I possess representative stages throughout the period of development which extends from this point on until the 'didermic blastocyst' is reached. In the present section we shall describe three blastocysts, which, although all of the monodermic

TABLE 2  
*Attached embryonic vesicles*

NO. OF SPECIMEN	DATE	SIZE IN MM.	OVARY	AGE OF MOTHER	FIGURES	REMARKS
307	10-22-12	0.516	L	2d year		Came off after 2 minutes in fixing fluid
328	10-28-12	0.468	R	Old		Came off immediately in fixing fluid
339	11-15-12	0.425	L			Came off after 2-3 mins. in fixing fluid
340			L	2d year	15,48	Came off immediately in fixing fluid
316			L	Old	16,31 43-46	
332	11- 2-12	0.500	L	2d year	7,49,50	Measurement across widest points
233	10-30-11	0.340	R	Old	8,32,51 52	
329	11- 2-12	0.312	R	Old	53	
289	10-15-12	0.336	L	Old	54,55	
298	10-19-12	0.440	L	Old	56,57	
234	11- 1-11	0.598	R	Old	19,33,58	
256	11- 4-11	0.944	L	Old	20,59	
247	11- 3-11	0.722	R	Old	1,21,22 34,60,61	
255	11- 4-11	1.000	L	2d year		
290	10-15-12	1.490	R	Old	2,23-26 62-65	
175	11-14-10	0.931	R	Old	27-29,31 66-69	
257	11- 4-11	0.909	L	2d year	3,30 70-74	
170	11-18-11	1.533	L	2d year	4,75-77	
226	10-30-11	1.367	L	Old	80-84	
276	11-21-11	3 x 5	L	2d year	5,78	
216	1-11-11	90	L	Old	79	Advanced stage, containing embryos 10 cm. long.

type, show interesting and significant differences. These will be described in the order of their development.

*Blastocyst No. 287.* This specimen measures 0.312 mm. in diameter, and is therefore larger than several others, but in point of development it is the youngest specimen in the collection. The inner cell-mass appeared both in the living and in the preserved condition as an opaque spot, the inner surface of which showed many elevations, caused by the protruding embryonic cells. In section the mass is seen to be made up of embryonic cells with relatively large, distinct nuclei (fig. 6). For the most part each cell is delimited by a cell wall; but here and there may be found one in which part of the wall has disappeared, or at least the membrane can not be made out with certainty. Another point of interest is the fact that there is a considerable difference in the size of the nuclei. Thus of the eleven nuclei shown in the median section (fig. 6) six are decidedly larger than the remaining five. I am unable to detect any difference in the staining properties of these two types of nuclei, although the cytoplasm of some of the cells which possess the larger nucleus takes a much paler tint than that of the cells with the small nucleus.

The embryonic knob measures 0.026 mm. deep by 0.055 mm. wide. If we regard the trophoblast of the median section of the blastocyst as the circumference of a circle whose diameter is equal to the diameter of the blastocyst, and using the width of the inner cell-mass as a chord, then we may express the size of the mass in the terms of degrees and minutes, as measured on the circumference by the subtended arc. In this particular specimen the diameter was 0.312 mm., and since the diameter of the inner cell-mass was 0.055 mm., it covered an arc on the circumference equal to  $20^{\circ} 18'$ . The embryonic mass is composed of about 75 cells, as determined by a count of the number of nuclei. They are tightly pressed up against the trophoblast, from which, however, they are sharply cut off by the under surfaces of the trophoblastic cells.

*Blastocyst No. 310* measured 0.360 mm. in diameter, and is therefore larger than the preceding, and that it is also further developed is evidenced by the fact that there are 136 cells in the embryonic

knob. In the whole condition the knob appeared as a conical-like elevation extending up from the trophoblast. The surface of the mass was uneven, due to the projecting embryonic cells (fig. 36).

In detail, the embryonic knob is composed of a central core of protoplasm surrounded by nuclei (fig. 7). Some of the nuclei lying towards the free surface of the mass are completely delimited by the cell-membranes, but in the majority of cases that portion of the cell membrane which is directed towards the center of the mass has faded out. Some of these incomplete cells have two nuclei. In the region of the trophoblast the cells have lost all traces of membranes, but Rauber's portion of the trophoblast retains its distinctiveness from the underlying embryonic mass.

While the embryonic knob of the preceding blastocyst presented when viewed from above, a perfectly sharp contour, the knob of this specimen, on the contrary, showed many ray-like protusions extending out from the base of the cone along the trophoblast. Thus the picture revealed in the upper view of such a mass is that of a many pointed star. The spreading of the inner cell-mass would seem to be accomplished by the migration of the cells from the base. They creep out with pseudopod-like processes, and can frequently be seen in the sections. On the left side of the median section (fig. 7) one of these processes is seen pushing out along the under side of the trophoblast, and on the right of the same section another cell is in the act of beginning a similar migration. In consequence of this spreading, the diameter of the embryonic mass has increased to 0.090 mm., as against 0.055 mm. in blastocyst No. 287; and the arc on the circumference covered by it is  $28^\circ, 57'$ .

*Blastocyst No. 335.* In many respects this is one of the most interesting of all the early blastocysts in the collection. It is easily the smallest, since it measures but 0.220 mm. in diameter; and as compared with No. 310, is less than two-thirds the size. Nevertheless, the differentiation of the embryonic knob is distinctly more advanced than that of the preceding blastocyst. What explanation are we to offer then for this apparent disparity in size? Is this variation in size of the vesicles to be correlated with a difference in the size of the undivided egg? I think not.

The explanation is rather to be sought in the condition of the trophoblast. If the median sections of blastocysts Nos. 310 and 335, which have been photographed at the same magnification (figs. 36, 38) be compared, it is at once evident that, while the trophoblastic cells in 335 are unchanged, remaining relatively thick, as they must have been at the close of cell division, those of 310 are very much attenuated. The nuclei have become widely separated by the stretching of the wall, which in turn has become so thin in places that it appears as a delicate line in the photograph. Evidently this enormous increase of the blastocyst is the result of the accumulation of fluid within the cavity of the vesicle, and the consequent stretching of the wall. It is a fact worthy of note that one never finds the trophoblastic cells undergoing division while the blastocyst is free within the uterine cavity. The conclusion to which we must come is that the accumulation of fluid in the cavity of the vesicle must, at least in some cases, go on quite independently of the differentiation of the embryonic mass.

Turning to the detailed structure of the embryonic region one finds that the inner cell-mass is flattened out until it has become a lens-shaped structure measuring 0.024 by 0.115 mm., and covering an arc on the circumference equal to  $63^{\circ}, 1'$ . There has also been some increase in the number of embryonic cells, the knob now showing 166 cells.

The point of greatest significance and interest concerns the two types of cells of which the embryonic mass is composed. In the two preceding blastocysts there were two sizes of nuclei observable and here not only does this same disparity still exist, but there is also a corresponding difference in the cells themselves. Thus there are clearly two types of cells, a large cell in which the nucleus is large, and a smaller cell with a comparatively small nucleus. In this particular specimen the small cells have a general distribution throughout the mass, and appear either singly, or in groups of two or more. The ratio of the small cells to the larger is about as one is to three. Thus in the median section there are fourteen large cells and five smaller ones. The latter are in three groups: a single cell lying on the surface at the extreme left (fig. 8 *a*), a pair situated some distance to the right of this (*b* and *c*), and a second pair

lying about one-third the distance from the right end of the section (*d* and *e*). In the first pair one of the cells borders on the under surface of the mass, while the other is wedged in between two large cells. In the other pair one of the components also comes to the under surface and the other is in contact with the trophoblastic cells.

I interpret the condition here to mean that the nuclei of the small cells are identical with the smaller nuclei of the preceding stages, or else the progeny of such nuclei.

The size relation of the different cells is not the only evidence which indicates that the embryonic mass is gradually differentiating into two types of cells; for the small cells have begun to take on a slightly deeper tint of stain than do their larger fellows, and this difference in the staining capacity of the two types of cells becomes more and more evident as development progresses, until finally it becomes one of the most striking features of the armadillo blastocyst.

## *2. The didermic stage*

The changes which we have just observed foreshadow the transformation of the monodermic blastocyst into one of the didermic type; that is to say, the differentiation of the inner cell-mass into its two primary components, the embryonic ectoderm and entoderm. We have seen that the inner cell-mass gradually differentiates into two rather distinct types of cells, which differ from each other both in size and in staining properties. The bulk of the mass is composed of large, faintly staining cells which are more numerous in that part of the mass which is situated towards the trophoblast. The other type of cell is much smaller than the preceding, takes the stain much more readily, and has a sharply defined outline. These smaller cells are at first evenly distributed among the larger (except in a few cases to be noted later), but later become collected toward that side of the inner cell-mass which borders on the cavity of the vesicle. Subsequently they become split off from the lower side of the mass to form the entoderm. In speaking of these cells in my preliminary paper I make the following statement.

There are, however, two distinct types of cells. The bulk of the mass consists of rather large, ill-defined cells, the cytoplasmic and nuclear portions of which do not stain well. Scattered among these are smaller cells, which are found much more abundantly in that part of the mass lying towards the cavity of the vesicle. The smaller cells are characterized by their sharply defined outlines and by the ease with which they take the stain. There is considerable evidence to indicate that the smaller cells are gradually undergoing a segregation from their larger fellows to form the hypoblastic layer of the vesicle. At any rate the hypoblast upon its completion possesses cellular elements that very closely simulate the smaller cells of the earlier stages.<sup>3</sup>

This statement was made before I had had an opportunity of examining Hill's ('10) excellent paper on the early development of the 'native cat,' *Dasyurus viverrinus*. In this paper Hill shows in the clearest possible way that the entoderm arises in a manner quite similar to that in the blastocyst of *Tatusia*, and I shall therefore briefly state his general results on the point under discussion. It will be recalled that in the 16-cell stage the blastomeres are arranged in two superimposed rings of eight cells each. The eight upper cells, which are smaller than the eight lower, are destined to produce the formative or embryonal region of the blastocyst wall, while the eight lower cells will give rise to the non-formative or extra-embryonal region. In accordance with the characteristic mode of development in the marsupial, no morula stage is formed in the egg of *Dasyurus*, but the blastomeres proceed directly to form the wall of the blastocyst. This is brought about through the division of the blastomeres of each ring and their gradual spreading toward the opposite poles, on contact with the inner surface of the sphere formed by the zona and the shell-membrane. The daughter blastomeres continue to divide and eventually produce a complete cellular lining to the zona sphere, constituting the unilaminar wall of the blastocyst. The wall remains in this unilaminar condition until the blastocyst attains a diameter of 4 to 5 mm.

Hill here draws the most fundamental conclusions of his paper, pointing out that the formative or embryonal region, which from the first possesses no covering of trophoblast (i.e., Rauber's layer),

<sup>3</sup> Loc. cit., pp. 369-370.

is the homologue of the inner cell-mass of the eutherian blastocyst; and that the non-formative region is the homologue of the hypoblast of the eutheria and of the extra-embryonal ectoderm of the sauropsida and monotremata.

After the blastocyst has reached a diameter of from 4 to 5 mm., two distinct varieties of cells can be recognized in the unilaminar wall of the formative region. Hill regards this as the crucial stage in the formation of the primary germ layers, as it marks the transition from the unilaminar to the bilaminar condition. We may quote from Hill's summary such paragraphs as give a résumé of his account of the development of the entoderm.

The formative region, unlike the non-formative, is constituted by cells of two varieties, viz.: (i) a more numerous series of larger, lighter-staining cells destined to form the embryonal ectoderm, and (ii) a less numerous series of smaller, more granular, and more deeply staining cells, destined to give origin to the entoderm and hence distinguishable as the entodermal mother-cells.

The entodermal mother-cells, either without or subsequent to division, bodily migrate inwards from amongst the larger cells of the unilaminar wall and so come to lie in contact with the inner surface of the latter. They thus give origin to the primitive entodermal cells from which the definitive entoderm arises. The larger passive cells, which alone form the unilaminar wall after the inward migration of the entodermal cells is completed, constitute the embryonal ectoderm.

The entodermal cells as well before as after their migration from the unilaminar wall are capable of exhibiting amoeboid activity and of emitting pseudopodial processes, by the anastomosing of which there is eventually formed a cellular entodermal reticulum underlying, and at first coextensive with, the embryonal ectoderm.

The entoderm is first laid down below the formative or embryonal region of the blastocyst; thence it extends gradually by its own growth round the inner surface of the unilaminar non-formative region so as to form eventually a complete entodermal lining of the blastocyst cavity. In this way the blastocyst wall becomes bilaminar throughout.

Thus it will be seen that, contrary to the generally accepted view of mammalian embryologists, the entoderm of *Dasyurus* does not arise by delamination, but through an inward migration of differentiated entodermal mother-cells from among the ectoderm cells of the embryonic region. The independent discovery of a similar method of entodermal formation in the armadillo is of more than ordinary interest, and calls for a detailed account of the process in

this eutherian mammal. Before taking up this account, it should be pointed out that the blastocyst of *Dasyurus* presents an unusually favorable opportunity for the study of the origin of the entoderm. The large size of the vesicle, together with the unilaminar condition of its wall, makes possible the preparation of the embryonic region as whole mounts, by the means of which detailed observations can easily be made. The eutherian blastocyst, on the contrary, does not admit of satisfactory whole mount preparations, owing to the fact that the embryonic region is more than a cell thick. In the blastocyst of the armadillo this is particularly true, as the wall of the formative or embryonal region is about four cells thick. Nevertheless, the evidence gathered from the study of sections, which we shall now present, is convincingly in favor of the view that the entoderm arises by the means of migrating cells, and does not favor the commonly accepted idea that it arises by a delamination, that is, by the splitting off of the lower layer of cells from the embryonic knob.

*Blastocyst No. 244* is next to the smallest blastocyst secured, measuring but 0.263 mm. in diameter, and, when taken, was on the point of entering the uterine cavity from the left fallopian tube. At this time it was distinctly spherical in outline, with an embryonic spot, having a slightly irregular margin and covering an arc  $52^{\circ} 58'$ . The specimen is described here, not only because it shows a continuation of the changes observed above, but also for the reason that it is remarkably well preserved, and consequently will give more than ordinary confidence to any interpretations which may be based upon its structures.

The embryonic spot, which measured 0.025 mm. deep by 0.100 mm. in diameter, was too thick to permit the determination of the details of structure from a study of the living specimen; but in the sections the details stand out brilliantly, and prove beyond a peradventure that there are two types of cells composing the embryonic mass. The entodermal cells, as in the case of specimen No. 335, are apparently quite generally distributed throughout the inner cell mass. The section passing through the center of the mass is typical in that it shows all of the essential features, and especially the relation which exists between the two elements con-

stituting the embryonic spot (fig. 9). There are six entodermal and thirteen ectodermal cells, or rather ectodermal nuclei, for in some cases two or more of these nuclei are included within a single cytoplasmic mass. No entodermal cells have been found which show more than one nucleus.

The entodermal cells in the section are located in three regions, similarly to those of the preceding figure. On the extreme left the single cell (fig. 9, *a*) is easily distinguishable from its adjacent, binucleated, ectodermal fellow. It gives evidence of beginning to spread out beneath the binucleated cell. To the right of this is a group of three entodermal cells (*b*, *c*, and *d*), one of which comes to the lower surface, while the other two lie one above the other, well within the mass. These cells are darker than the neighboring ectodermal cells, but the difference in the size of the nuclei that was so striking a feature of the preceding stage is here not so marked. In fact, the nuclei of some of the ectodermal cells are smaller than those of the entodermal ones. It must be kept in mind, however, that in these thin sections only the tip or at least a small portion of a large nucleus may be visible in a given section. A study of the preparation shows that on the average the entodermal nuclei are smaller than the nuclei of the ectodermal cells. The remaining entodermal cells constitute a pair situated about in the middle of the right half of the section (fig. 9, *e* and *f*). Both of these cells are on the surface of the mass. Their cytoplasmic and nuclear portions do not stain so deeply as in the other entodermal cells, but still more deeply than in the case of the ectodermal cells.

The affinity of the entodermal cells for the stains evidently lies in the nature of the protoplasm itself. In the ectodermal cells, both in the cytoplasm and nucleus, the structural configuration of the protoplasm is of a more open mesh-like character than that of the entodermal cells, in which it may assume a finely granular appearance.

The conditions observed in this and the other sections of the series is interpreted to mean that the more deeply staining cells are entodermal, all of which will eventually migrate to the lower surface of the embryonic mass to form the characteristic entodermal layer of the mammalian blastocyst. The cells marked *c*, *e*, and *f*

are in the act of migrating to the surface. The cells lying within the mass (*b* and *d*) are rounded in outline, while those in the act of coming to the lower surface are invariably elongated in the direction of migration.

The evidence obtained from a study of these sections indicates that each group of two or more entodermal cells has, in all probability, arisen from a single primary cell, or entodermal mother-cell (using Hill's terminology). Furthermore it suggests that the primary cell may, from the first, be situated on the surface; and consequently will undergo no migration; or it may migrate to the surface without having undergone division; or again, divisions may have come in before the migration occurs.

*Blastocyst No. 296* came from the proximal part of the left fallopian tube, and measured 0.376 mm. in diameter. The embryonic spot had a very even outline and measured 0.161 mm. across. It is distinctly thicker than the last specimen, averaging about 4 cells, exclusive of the trophoblast (fig. 10). The arc covered by the embryonic spot is 50°, 42'.

In every way the blastocyst is more advanced than any we have so far described. It is not only larger, but also shows a higher state of differentiation. One feature in particular, although not entirely unique since it has been observed in one or two other cases, is nevertheless worthy of mention. This is the presence of faint structures on the outer surface of the trophoblast (fig. 37). These were observed in the preserved egg and were naturally taken to be the follicle cells, which sometimes adhere to the ovulated mammalian egg and persist for some time; but in section they are seen to be protrusions or exudations from the trophoblastic cells, and are probably formed at the time the egg is fixed.

The number of entodermal cells in the median section is thirteen as against sixteen ectodermal cells. This would seem to indicate that there has been a great increase of entodermal cells, but in several of the other sections they are very much less numerous, owing to the fact that the median section passes through the principal groups of entodermal cells.

The difference in size between the ectodermal and entodermal cells is very obvious (fig. 10), and, as compared with the preceding figures, stands in sharp contrast.

The distribution of the entodermal cells is interesting and instructive. Except for a single cell (fig. 10 *m*) the entire upper half of the embryonic mass is free from them; and certainly this suggests that these cells are gradually passing towards the lower surface. Already nine of the remaining twelve cells have reached the lower surface. The position of the twelve lower cells indicates that they probably came from five different sources (five entodermal mother-cells) as follows: cell *a* is in the act of migrating to the surface; cells *b–e* have had a common origin; likewise cells *i–k* have probably come from a single mother-cell; and finally, cell *l* gives evidence of once having occupied a position against the trophoblast between the two ectodermal cells situated farthest to the right in the section. It is now clearly in the act of migrating out from between these two cells.

I have already stated that cell *m* is the only entodermal element situated in the upper half of the mass. There is no way of determining whether this cell will also migrate to the lower surface. A pseudopodial-like process from its lower border is pushed in between two ectodermal cells, and suggests at least that it is about to move down. Furthermore, in the later stages, when the entoderm is completed as a distinct layer, no such cell as *m* is found within the ectoderm.

The cell which lies just above *e* has caused me considerable difficulty in attempting to determine to which of the two categories it belongs. Its relatively small, deeply staining nucleus closely resembles those of the entoderm, but its faintly tinted cytoplasm, together with its square-like outline, are sufficient to place it among the ectodermal cells.

A word here as to the changes occurring in the size and thickness of the inner cell-mass may be said. These changes can be seen by comparing figures 6 to 11. At first the inner cell-mass is composed of a group of spherical cells, but, as development progresses, the mass becomes flattened out against the trophoblast, until finally it forms a circular plate of cells about two deep. This period is then followed by one in which there is a distinct increase in the thickness of the mass, due evidently to the multiplication and growth of the cells. Growth and multiplication of the cells con-

tinues until the maximum thickness is reached in such specimens as No. 296 (fig. 10), when the embryonic spot again begins to spread and continues to increase in diameter until finally the mass is reduced to about two cells in depth (fig. 11).

This process of migration of the entodermal cells to the lower surface of the embryonic knob and their subsequent peripheral movement along the inner surface of the trophoblast must take place by amoeboid activity. Indeed, the evidence for this conclusion is irresistible. If one examine in the living condition a stage somewhat more advanced than No. 296, one finds that numerous pseudopod-like processes are radiating out in all directions from the embryonic spot. In many instances the connection of these processes with some of the outlying entodermal cells is clearly discernible.

In order that a photographic record of this phenomenon might be made, glycerin jelly mounts were prepared of several unstained blastocysts which exhibited it. A photograph of such a preparation is shown in figure 40. In the enlarged view of the embryonic spot of the same specimen (fig. 39), the pseudopodia are particularly clear and striking. The high power of the microscope was focused so as to bring the entoderm as sharply as possible into view and while the rather thick ectoderm of the central area obscured the true condition of the entoderm here, yet the peripheral portions are brought out sharply. A small area of these anastomosing processes from the lower border of the embryonic area is sketched in figure 13. Some of the pseudopodia have sharply pointed ends, others have rather blunt ends, and still others have flattened terminations. The processes from two or more cells frequently anastomose and form a fenestrated structure. In sections also the pseudopodia can be demonstrated, especially when the section happens to cut one of them lengthwise (fig. 14). However, it is from the study of the living material and glycerin jelly preparations that one obtains the most convincing evidence of these pseudopodia, and of the rôle they play in the formation and migration of the entoderm.

The segregation of the entodermal cells from the ectodermic mass is completed by the time the vesicle has attained a diameter

of about 0.430 mm. At this time the embryonic spot has not reached its maximum expansion, that is, it has not completely flattened out. Consequently in section the entoderm forms a slightly curved line, due to the bulging out of the mass of embryonic ectoderm (fig. 14). The ectodermal cells are large, relatively clear, and sharply cut off from the overlying trophoblastic cells. On the inner surface of the mass they are less sharply separated from the entodermal cells, which here and there send processes up between some of the bordering ectodermal cells. Such processes are undoubtedly the last remnants of the migrating entodermal cells to be withdrawn from the embryonic mass.

The condition of the entoderm is of much interest. It does not as yet form a complete sheet of cells underlying and coextensive with the embryonal ectoderm. In places the entoderm may be wanting for more than the width of a cell. Furthermore, on the right side of the sections of specimen No. 249 the entoderm is frequently wanting, indicating that it must have taken its origin from the left portion of the embryonal mass. In some of the earlier blastocysts this same fact was observed. The smaller, deeply staining cells, which give rise to the entoderm, were found to extend over not more than two-thirds of the embryonic mass. Finally, in the largest free blastocyst secured (No. 300) and one in which the entoderm is completely segregated, we find this same eccentricity of the entoderm. It was so evident in this specimen that I have gone to the trouble of making a special preparation for the purpose of demonstration by a photograph. The vesicle in question was slightly stained in eosin and imbedded in paraffin. Under the high power of the binocular microscope the non-embryonic hemisphere was carefully pared away with a sharp razor. The remaining hemisphere was dissolved out of the paraffin, stained in hematoxylin, and mounted in balsam, with the cut surface uppermost. Thus it was possible to get an unobstructed view of the entoderm, and since the embryonic mass had become completely flattened, the entodermal layer lies in a single plane. The exposed sheet of entoderm was then studied and photographed (fig. 42).

The center of the embryonic spot lies about five millimeters below the center of the circular figure, and, since the entodermal cells are practically all in focus, it can be seen that, as a continuous layer, the entoderm covers only about the lower three-fourths of the embryonic spot. Over the other fourth only a very few entodermal cells are found, and these lie for the most part slightly to the left of the center. The other cells in this area which are slightly out of focus represent the exposed ectoderm, which is here only about one cell deep.

What does the excentric position of the entoderm mean? The observation of this phenomenon in some four or five blastocysts doubtless furnishes too meager evidence upon which to base any fundamental conclusion. Nevertheless one can not resist the temptation to suggest that we may have here a key to the much mooted question of gastrulation in eutherian mammals. For if it could be shown that the origin of the entoderm is confined to a definite area of the embryonic mass, the center of such an area might be regarded as corresponding to the region of a blastopore, regardless of whether or not this spot later became perforated by an actual opening or evanescent blastopore. Hubrecht ('02 '05 '08) has argued that the didermic stage of the mammalian blastocyst is to be regarded as a 'gastrula'. He further states that we must separate the phenomena of notogenesis from the phenomenon of gastrulation. He expresses himself very clearly and concisely on this point in the last of the three contributions mentioned above in which he makes the following statements:

As soon as we separate the phenomena of notogenesis, such as we have found in all vertebrates—*Amphioxus* included—from the phenomenon of gastrulation, recognizing that the former follow upon the latter and bring about the formation of the notochord and the mesoblastic somites, the difficulties are considerably simplified.

Gastrulation is thus terminated in the mammalia when the didermic stage of the embryonic shield has come into existence. We have seen that this takes place not in consequence of any process of invagination but by means of a most unmistakable delamination of the entoderm, out of the embryonic knob.

This delamination gastrula of the mammalia generally enters upon the latter phases of ontogeny which will be described hereafter without the appearance of a distinct blastopore.<sup>4</sup>

<sup>4</sup> Loc. cit., p. 13.

Concerning the idea of separating the processes of gastrulation and notogenesis I believe that in the main we must agree with Hubrecht, although the close genetic continuity of these two processes must never be lost sight of, even in mammals, else the appearance of an evanescent blastopore in such forms as the hedgehog, *Tarsius*, rabbit, mole, shrew, and opossum would have little significance. But the unexpected discovery of migrating cells to form the entoderm in the blastocyst of the armadillo must deter us from speaking of the process of entoderm formation as one of delamination, for this term as used in mammalian embryology implies that the entoderm is differentiated from those cells of the inner cell-mass which happen to border on the cavity of the vesicle. So to regard the origin of the entoderm would be equivalent to accepting Driesch's ('93) aphorism; for it would amount to saying that the prospective value of one of these bordering cells "is a function of its position." Delamination is not therefore the correct term to employ in describing the mode of entoderm formation in the armadillo, for while it is true that the entoderm as a distinct layer is split off from the embryonic ectoderm, as we shall see later, yet prior to this so-called delamination there is an unmistakable migration of primary entodermal cells to the lower surface of the inner cell-mass, and this it seems to me is the fundamental step in the whole process of entoderm formation.

The final steps in the differentiation of the entoderm in the armadillo may be considered here, as we shall not have occasion again to refer to them. Following the conditions such as we have seen in figure 41 the entodermal cells spread out until they have formed a continuous sheet of cells beneath and coextensive with the embryonic ectoderm (fig. 47). During this change the entodermal cells become much flattened against the ectoderm. There is no evidence here, such as we have observed in the pseudopodia of earlier stages, to indicate that the entodermal cells at the margin of the embryonic area are pushing out further beneath the trophoblast. Even in the specimen shown in figure 42 the pseudopodia have practically all disappeared, except in the case of the upper marginal cells that border on the area of ectoderm still not covered by entoderm (fig. 12).

Measurements show that the entoderm has practically reached its maximum extension in such specimens as No. 311, in which the embryonic spot measures 0.020 mm. by 0.157 mm., and covers an arc on the circumference of  $36^{\circ}, 18'$ . It does not here reach much beyond the extreme limits of the ectoderm (fig. 11). This does not mean that the entoderm may not cover an actually great area, for it becomes attenuated through the expansion of the trophoblastic wall; but what is meant is that the entodermal cells do not push out any further along the trophoblastic wall. Consequently that part of the wall which lies beyond the limits of the embryonic area never becomes didermic, or bilaminar. Perhaps then it would be better to confine the use of the term didermic to the embryonic spot, and not apply it to the blastocyst as a whole.

It may not be amiss in conclusion to draw attention again to the very close similarity between the mode of origin of the entoderm in the armadillo and that of *Dasyurus*, as given by Hill. The similarity is indeed striking, especially if one consider the fundamental differences in the character of the walls of the two types of blastocysts. The thin wall of the formative region of the blastocyst of *Dasyurus* stands in sharp contrast to its homologue, the relatively thick inner cell-mass of the armadillo blastocyst, and yet in all essential features their modes of entoderm formation are practically identical.

#### ATTACHMENT OF THE BLASTOCYST

The implantation of the embryonic vesicle is a subject of much importance, and is treated elsewhere as fully as the material at hand warrants. At this point it is desirable to discuss briefly only the first step in implantation, or the attachment of the blastocyst.

A great deal of effort has been put forth to obtain the earliest attached stages, and, to date, four clear cases have been observed. Two other doubtful cases were seen. Unfortunately in each case the vesicle became detached from the mucosa upon placing the uterus in the fixing fluid or very shortly thereafter. In two of these the separation took place immediately, while in the other two from two to three minutes elapsed between the immersion of the

uterus and the loosening of the vesicle. However, these stages were studied under the high and low powers of the binocular microscope before the uterus had been placed in the fixing fluid, and it was therefore possible to make out most of the details of structure.

In general appearance these vesicles are not unlike the largest of those which were found lying free within the uterine cavity. The large polygonal trophoblastic cells are clearly discernible, and the embryonic area appears as a whitish spot lying directly in contact with the mucosa. In size, too, they are not larger than the more advanced free-vesicles.

These four vesicles were sectioned for microscopic examination, and in three of them a detailed study reveals nothing different from what we have already seen in the free stages; but in the fourth, which was one of the two that remained attached for the longest period, an important difference was observed. The change to which I refer involves the most essential part of the vesicle, the embryonic ectoderm, and consists of a thickening of that structure (fig. 48). It is certain that this increase is not due alone to a multiplication of cells, since mitotic figures are rarely found, but to a distinct rounding up of the entire embryonic ectoderm. In fact this change is the beginning of a process that will eventually transform the lens-shaped ectodermal mass into a ball-like structure.

In the study of these four vesicles particular attention has been paid to the area of trophoblast (Rauber's layer) which directly overlies the embryonic ectoderm, and which forms the seat of attachment. The trophoblastic cells of this area do not as yet betray any evident changes looking to the formation of the 'Träger.' We must conclude therefore that for a short time at least the vesicle is held to the mucosa by adhesion. It would seem that it simply 'sticks' to the uterine lining. Nor is there any evidence to show that there is a localized area within the attachment zone of the fundus to which the vesicle migrates before becoming attached. Any spot on the entire attachment zone may furnish a foothold for the vesicle, if one may judge from the collected data on the distribution of about twenty young attached stages. Apparently the vesicle adheres to the mucosa very soon after it

passes from the horizontal groove to the attachment zone; for it is almost invariably the rule that the vesicle is located on that half of the zone lying adjacent to the groove along which it had passed from the fallopian tube. Thus, in a pregnancy in which the left ovary holds the corpus luteum, the vesicle will be found to be attached at some spot on the left side of the attachment zone.

#### FORMATION OF THE ECTODERMIC VESICLE

In the preceding paragraphs we have referred to the change involving the embryonic ectoderm which takes place shortly after the vesicle becomes attached to the mucosa. This change is perhaps anticipated in the more advanced of the free blastocysts in which a few divisions of the ectodermal cells are taking place (fig. 47); but evidently does not express itself clearly until after the egg becomes quite well anchored to the uterine wall. As already intimated, the change consists in the transformation of the flat, circular plate of ectodermal cells into a spherical or ball-like mass (fig. 15).

The entoderm in this stage is recognized as a distinct layer, and, although no longer connected with the ectodermic mass by protoplasmic strands, yet is still in close contact with it. The entodermal cells which lie directly beneath the ectoderm become distinctly thicker than before, and accompanying this change is the appearance of mitoses (fig. 15).

The entodermal cells which lie beyond the limits of the embryonic ectoderm, differ from those lying beneath the ectoderm in two important respects. First, the nuclei of the cells remain small, thus indicating that the cells are not in an active state of division; and second, these cells are flattened out against the trophoblast, with which they are in very close contact. These cells form a narrow ring or annular zone around the margin of the ectoderm. This zone is of especial interest because it forms the axis about which the so-called inversion of germ layers revolves.

The transformation of the plate of ectoderm into a spherical mass results eventually in its entire separation from the trophoblast and its inclusion within an entodermal sac, thus leaving a

cavity between the Träger and the ectoderm. Material with which to follow the steps through which the blastocyst passes during this is not at hand, for there is here a slight gap in the series. However, I have been fortunate enough to obtain two blastocysts which show the condition immediately following the inclusion of the ectoderm. The two specimens are nearly of the same age, one being slightly more advanced than the other.

The younger blastocyst (No. 316) was found attached to a small leaf-like outgrowth from one of the folds of the placental mucosa. The outgrowth lay horizontal to the surface of the mucosa, and the vesicle had gained attachment to its under side, close to the edge. One side of the trophoblastic wall caved in during fixation, otherwise the vesicle is in an excellent state of preservation (fig. 16).

That portion of the trophoblastic wall which has caved in presents nothing different from what was observed in earlier stages, except that the cells are more attenuated, but the opposite wall of the vesicle has undergone a marked thickening, in addition to a great increase in its cellular elements. The cells have, therefore, changed in shape from a flattened squamous type to a distinctly columnar epithelium. All the thickened portion of the wall was originally in contact with the mucosa, but during the process of fixation the leaf-like fold of the mucosa has undergone a great deal of contraction and has shrunk away from the trophoblast, as is evident from its folded appearance. Evidently the thickened trophoblast owes its existence to contact with the mucosa, which in some way specifically stimulates the cells to a striking activity, as shown by the rate of division and change in shape. The specific reaction between the trophoblast and the mucosa must be limited to that portion of the uterine epithelium which covers the placental area, otherwise the wall of a free vesicle would give evidence of response before the blastocyst had reached this area.

It is also a point worthy of note that the columnar cells send out pseudopod-like processes, which eat into the mucosa, giving it a serrated appearance in section. That the placental trophoblast has therefore a specific action on the trophoblastic cells can

not be doubted, and this effect seems to be limited entirely to the region in direct contact.

The sections are cut somewhat obliquely, and hence the section passing through the middle of the embryonic ectoderm does not cut the region of the primitive placenta, or point of attachment (fig. 16). Consequently the exact relation of the entoderm to the other parts of the blastocyst can only be made out by referring to several of the sections of the series. In plate 2 is shown a series of four photographs which will demonstrate this relation.

In figure 43, which represents the section from which the drawing was made (fig. 16), the entoderm is a well organized layer passing around the sphere of ectoderm. On approaching the lower side of the ectoderm the entoderm at each side passes laterally and upwards to join on to the inner surface of the trophoblastic wall (fig. 16, *x*). The layer of entoderm lying directly beneath the ectoderm belongs to that portion which connects the entodermal sac with the left wall of the trophoblast. A few sections to the right in the series the lower layer of entoderm disappears, except a small group of cells (fig. 44). In this section the entoderm, on leaving the ectoderm, passes outwards and upwards as before, to join the inner surface of the trophoblast. Aside from the group of entodermal cells lying directly beneath it, the embryonic ectoderm is open below to a cavity which is bounded beneath by the placental portion of the trophoblast, and which represents what is later to become an extraembryonic cavity. In sections still farther to the right, at a point where the section cuts just the tip of the ectoderm (fig. 45), the extraembryonic cavity is almost free from cells of any kind. This section passes through the center of the Träger (fig. 31). Beyond the limits of the ectoderm (fig. 46) we see nothing but a line of entodermal cells, which represents the right lateral portion of the entodermal layer as it passes outwards to unite with the trophoblast on this side of the blastocyst.

To sum up: The conditions revealed in the series of photographs shows that the ectoderm, upon assuming a spherical form pushes up into the cavity of the blastocyst, carrying before it the well established layer of entoderm, and creating behind a cavity which gives rise to the extraembryonic cavity, and which will

subsequently become lined with a layer of mesoderm. The condition here presented reminds one somewhat of that found in a corresponding stage of the blastocyst of *Pteropus edulis* (Selenka and Göhre '92), except that in the case of the latter the entoderm continues around the inside of the trophoblast, forming a complete, inner layer to the blastocyst.

In the older of the two blastocysts (No. 332) the sections pass exactly parallel to the median axis of the vesicle, and consequently the relation of the different parts of the embryo is clearer than in No. 316. The general conditions of the trophoblast are much the same in the two specimens, except that at one point on No. 332 there is a knot of cells (fig. 17 *k*) which, in the living condition, fitted into a corresponding crypt in the mucosa. In later stages we shall see further evidence of similar knots, which represent points on the trophoblast that have been specifically stimulated to cell proliferation.

The relation of the entoderm to the embryonic ectoderm is remarkably clear in this preparation (fig. 17). In contrast with the preceding blastocyst, the entoderm of this specimen has undergone one important change, in that it has folded in beneath the ectoderm, forming all but a closed entodermal sac. Only a small pore-like opening (fig. 17) remains to place the extraembryonic cavity in communication with the cavity of the entodermal sac. On the right-hand side (or lower side, owing to the inclination to the right of the blastocyst) a fusion has taken place between the two layers of the entoderm and a portion of the Träger; but this fusion, especially with the Träger, covers a very small area, as it no longer exists in the sections a short distance to either side of this one. The loop of entoderm which lies between the fused area and the pore (fig. 49) is probably comparable to the group of cells situated in a similar position in the other specimen. The loop of cells is especially clear in figure 17.

Returning to a fuller consideration of the ectodermal sphere, we see that even in so young a stage as that of No. 316 it is no longer a solid mass of cells, as must have been the case at first, but in the central portion there are three relatively large and distinct besides several smaller, less distinct vacuoles. In fact, the

entire core of the ectodermal sphere becomes honeycombed by these vacuoles, which later unite. In blastocyst No. 332 the union of the vacuoles has already made considerable progress (figs. 49, 50). Eventually there is produced a large distinct cavity within the ectodermal mass, thus transforming it into a true vesicle.

I obtained one vesicle which clearly represents a further advance in the progress of vacuolization, and yet one in which the completed stage of the vesicle has not been attained. Unfortunately the specimen became slightly crushed in the course of transportation from the field to the laboratory, after it had been fixed and partially hardened. I therefore deem it unsafe to base any definite conclusions upon its structures; but simply give a photograph of one of the sections (fig. 53), which, in a measure at least can be understood after we have considered a normal specimen of a little later stage.

In figure 51 is shown a section of a stage at the completion of the ectodermic vesicle. The specimen consists of the following structures: (1) an outer layer of trophoblast, which on the lower side has become modified into the primitive placenta; (2) an incomplete entodermal sac which is connected laterally with the trophoblast; and (3) an ectodermic vesicle.

The trophoblast is composed of a single layer on the upper or free surface (fig. 18), but towards the base it thickens into two layers of cells, especially on the right side of the figure. The trophoblast, now in contact with the mucosa, has greatly extended its area, as compared with that of specimens Nos. 316 and 332. Since it is intended to devote an entire chapter to the subject of placentation, we shall not give here any further consideration to the lower layer of trophoblast, which is of course concerned with placenta formation.

The entodermal sac is, as already stated, incomplete, remaining open on the side turned towards the mucosa. The cells at the point where the layer turns out to join the trophoblast are relatively thick, while those of that portion of the entoderm which passes over the ectodermal vesicle have undergone no important change. In the extraembryonic cavity are found a few scatter-

ed cells which are, for the most part, of entodermal origin, but which will soon disappear.

The entodermic vesicle is completely developed. Its upper or embryonic side is two or three cells thick, while its lower side is but a single cell deep. On this side is a small opening or pore which places the amniotic cavity in communication with the extraembryonic cavity. This pore is found in but four sections, and no other vesicle shows it, thus leading one to suspect that its existence is more or less accidental, due to the manner in which the vacuolization took place. In blastocyst No. 332 the vacuolization occurs towards that side of the ectodermal mass which is nearest to the mucosa, and in the present specimen we may suppose that the excentric position of the vacuolization has resulted in perforating the lower side of the vesicle.

There is, of course, another very plausible explanation, namely, that the opening appears in all of the vesicles, but soon closes thus accounting for the fact that it is never seen in the older stages. This view receives support from the work of Fernandez ('09) on *Mulita*. The youngest stage secured by Fernandez is about in the same state of development as this specimen of the Texas armadillo, and presents the same structural relations. Fernandez states that the cavity of the ectodermal vesicle and what he calls the 'Träger cavity' are connected by a small pore which he compares to the 'Verbindungsröhré' in the mouse (Melissinos '07). His 'Träger cavity' represents the same space that I have called the extraembryonic cavity in *T. novemcincta*. I have so named this cavity because later, when it becomes lined with mesoderm, it is recognized as a true exocoelome. It should be pointed out here that what Fernandez terms the Träger cavity in his second youngest stage (Fernandez '09, text figure 2) I have regarded as an artifact (for reasons to be presented later) and consequently it can not be compared with the similarly named cavity of his youngest stage.

In this mode of producing a vesicle of the ectodermal sphere is to be recognized an amnion formation through a process of vacuolization; for the entire subsequent history of development demonstrates that the cavity thus formed is an amniotic cavity.

The cavity has been previously termed the 'common amniotic cavity' (Fernandez '09, and Newman and Patterson '10), because it is common to the amniotic connections of the four embryos which later take their origin from the ectodermal vesicle.

It is evident from this that in the mode of amnion formation the armadillo is to be classed with that group of mammals in which the amniotic cavity is from the first an enclosed space, and never has a free communication with the space outside the trophoblast. The cavity is always intra-trophoblastic (Hubrecht '08), and consequently no folds ever arise to delimitate it, for this is not necessary. The armadillo can therefore be added to Hubrecht's ('08) compiled list of mammals, in which the amniotic cavity arises within the ectoderm and remains a closed vesicle. His list is as follows: "Cavia and other rodents, *Pteropus*, *Galeopithecus*, *Erinaceus*, *Gymnura*, monkeys and man.<sup>5</sup>

#### ORIGIN OF THE EXTRAEMBRYONIC MESODERM OR MESOTHELIUM

The origin of the mesoderm in mammals is a problem which has given rise to much difference of opinion among embryologists, but it is not necessary here to enter into this controversy. The first appearance of the mesoderm in the armadillo blastocyst is seen in connection with the cavity which lies between the ectodermal vesicle and the placenta, or what has been termed above, the extraembryonic cavity.

The entoderm, at the angle where it parts company with the ectodermal sphere to join the trophoblast (fig. 51), gives rise to a few cells which frequently become scattered throughout the extraembryonic cavity. There is good reason for believing that most of these cells undergo disintegration pari passu with the development of the ectodermal vesicle. At any rate there comes a time when the exocoelomic space is essentially free from such entodermal cells (fig. 54). In some of the sections of this series a few of these cells are still found within the cavity. Thus in figure 55 there are four of them, two lying some distance below the ectoderm and slightly to the left of the center, and two situated against the

<sup>5</sup> Loc. cit., p. 71.

under side of the ectodermal vesicle, at a point where the embryonic and amniotic portions of the vesicle meet on the left side. All such straggling entodermal cells show signs of decadence, and without doubt play no rôle in the development of the mesoderm.

The first evidence of mesoderm formation is also found in this same vesicle, and is indicated by the beginning of a process of proliferation involving the ectodermal cells which lie at the point or angle where the entoderm parts from the ectodermal vesicle. In figure 55 one of the proliferated cells, which has just been set free, is seen on the right, slightly removed from the angle. Only a very few such cells are found about the edge of the vesicle, but the presence of mitotic figures in this general region of the ectoderm indicates the approach of a rather profuse proliferation of mesodermal cells.

In another vesicle, somewhat larger than the preceding, the formation of the mesoderm has made rapid progress. The cells are proliferated in clusters, and soon develop into small vesicular structures (figs. 56, 57). In this particular specimen there are about eight small mesodermal vesicles, but it was not possible to determine whether their origin was confined to one or more localized regions of the ectodermal vesicle, or centers of active proliferation. The preparation shows every stage in the formation of vesicles. In most instances the cluster of cells is set free from the ectoderm before a cavity appears within their midst; in others, the cavity arises while the cells still retain a connection with the ectoderm. In all cases the smaller mesodermal vesicles gradually fuse together to form larger and larger cavities, until finally the entire space lying below the ectoderm is lined with a mesodermal layer.

While I was unable to determine any definite localized regions of mesoderm proliferation in this specimen, in all the older stages the mesodermal vesicles fuse in such a way as to produce two main vesicles. For example, specimen No. 234 has two well differentiated mesodermal vesicles, which are unequal in size (fig. 19). The larger one on the left-hand side is composed of a typical mesodermal layer. In this region it is free from any connection with the ectodermal vesicle, but in other sections it not only has such connections, but is also united to other smaller mesodermal ves-

cles, with which it is undergoing fusion. The smaller vesicle is similar in its general structure to the larger one, but retains a distinct connection with the ectodermal vesicle.

The relation of the mesodermal vesicle to the rest of the chorio-ionic vesicle is of great interest, and can be illustrated by explaining the manner in which the sections have been cut. In each of the specimens represented in figures 51 to 61 the sections have been cut so that their plane is parallel to the plane passing through the 'horizontal grooves' of the uterus and perpendicular to the surface of the mucosa (fig. 21). We thus see that the right and left mesodermal vesicles lie on those sides of the blastocyst which are turned towards the right and left openings of the fallopian tubes, respectively.

In figure 19 the mesodermal vesicles lie to the right and left of the center of the blastocyst, and, as we shall see later, hold the same orientation as do the two primary buds of the embryos.

Figure 59 shows a median section of a vesicle in which the two mesodermal vesicles have expanded until they occupy the entire extraembryonic cavity; but the double partition made by the approach of their adjacent sides does not allow a communication of the two cavities (fig. 20). However, this condition does not exist throughout the entire series of sections, for in many places a portion of the partition has already broken down. Eventually it will entirely disappear. This final condition in the formation of the extraembryonic mesoderm is soon reached, and the single layer of flattened mesodermal cells then completely lines the space below the ectoderm, conforming to the various irregularities of its bounding walls (fig. 22). The new cavity thus formed and lined with the mesodermal epithelium may now be called the extraembryonic coelome.

#### COMPARISON WITH OTHER FORMS

At this point it is well to compare briefly the armadillo blastocyst with that of other forms. The early development of the armadillo parallels most closely that of the mammals in which the so-called inversion of germ layers is found. Fernandez ('09) has pointed out that certain stages of the South American armadillo

Mulita are comparable to corresponding stages of the mouse, as figured by Melissinos ('07); and, while a similar comparison may be made between the mouse and the Texas armadillo, nevertheless, a closer similarity exists between the early stages of the frugiverous bat *Pteropus* and this armadillo. Thus one of the youngest stages of *Pteropus* figured by Selenka and Göhre ('92 pl. 41, fig. 4) is strikingly like the blastocyst shown in figure 17 of this paper; for in each case the embryonic ectoderm has separated from the trophoblast to form a spherical mass, which has become included within the entoderm.

The principal feature in which they are dissimilar is seen in the extension of the entoderm. In *Pteropus* the entoderm completely lines the cavity of the blastocyst, forming an epithelial lining for the yolk-sac. In the armadillo the entoderm extends out along the inner side of the trophoblast for only a short distance from the ectoderm, and at most never covers an area of over 80° on the circumference of the blastocyst. Consequently a closed epithelial sac of entoderm is not formed, and the yolk-sac cavity is bounded on the non-embryonic side by a single layer of trophoblastic cells, or chorionic ectoderm (fig. 19).

The similarity between *Pteropus* and the Texas armadillo is not confined to this early period, but is also seen in later stages; especially is this true with reference to the formation of the amniotic cavity. In each, the solid sphere of ectoderm becomes hollowed out through the disintegration or vacuolization of the core to form the primary amniotic cavity (*cf.* fig. 18 with fig. 6 of Selenka and Göhre). Finally, in the condition of the mesoderm the two forms show several points of similarity.

In the blastocysts of the mouse and of the armadillo are also to be seen many points of similarity, though the resemblance is here less striking than in the preceding case. The figures of Melissinos ('07) are very similar to several of the stages shown in this paper. His figure 31 shows a stage directly comparable to our specimen No. 311 (fig. 11); and his figures 33 and 34 illustrate the manner in which the embryonic ectoderm is pushed out into the general cavity of the blastocyst, carrying before it the visceral layer of entoderm. In the armadillo I have not succeeded in ob-

taining a stage which corresponds exactly to the stage of the mouse shown in figure 33 of Melissinos, although specimen No. 340 (fig. 15) may be compared with it. Figure 34 of Melissinos and my figure 19 can be directly compared, especially if it be kept in mind that the parietal layer of yolk-sac entoderm is incomplete in the armadillo. In the mouse the embryonic ectoderm is borne upon a mass of cells connecting it with the ectoplacental plate, and the separation of these two embryonic structures does not take place for some time. In the armadillo, on the contrary, the ectodermal mass early parts company with the Träger or ectoplacental region, thus giving rise to an extraembryonic cavity at a very early stage. It is this difference in the time of appearance of the extraembryonic cavity which renders difficult an exact comparison between the two forms throughout the subsequent history of development; and in support of this view we may cite the case of mesoderm formation.

We have seen that the mesoderm does not make its appearance in the armadillo blastocyst until after the extraembryonic cavity has arisen, and that upon arising from that portion of the ectodermal vesicle which is turned toward the placental region, it immediately develops into an epithelial lining membrane for this cavity, which is thereby transformed into a true extraembryonic coelome.

In the mouse, according to the account of Melissinos, the mesoderm is very early recognized as a mass of cells lying in a position somewhat similar to that of the mesoderm in the armadillo; that is, immediately ventral to the ectodermal mass, at the point of constriction between the embryonic ectoderm and the group of cells connecting it with the ectoplacental plate. Later, when the embryonic ectoderm and the ectoplacental plate become entirely separated, a cavity lined with mesoderm appears between these two embryonic structures. This cavity becomes then an extraembryonic body cavity (the 'mittlere Höhlung' of Melissinos). When this process is completed we are presented with a condition quite similar to that of a relatively late but corresponding stage of the armadillo (*cf.*, fig. 43 of Melissinos and my fig. 22). The essential difference lies in the fact that in the armadillo blastocyst there is no ectoplacental cavity; but even this difference later be-

comes lessened upon the formation in the armadillo of a distinct Träger epithelium, with a potential cavity lying between it and the invaded mucosa (fig. 23).

Other comparisons might be drawn between the armadillo blastocyst and those of other mammals, but this is not necessary. Sufficient evidence has been presented, I believe, to establish the fact that the early stages of the armadillo give a history corresponding in its general outlines to the development of an egg which in other forms produces but a single individual. The differences noted are no greater than would be expected to exist between mammals as widely separated as the armadillo and the bat or mouse.

#### ORIGIN OF PRIMARY BUDS

In the late stages of development it has been demonstrated (Newman and Patterson '10) that the four embryos of an armadillo litter are arranged within the single chorion in two pairs, which hold a very definite orientation with reference to the uterine axes. Thus it was found that one embryo always occupied that portion of the chorionic cavity lying adjacent to the dorsal wall of the uterus, one holds a ventral position, and the other two lie to the right and left sides, respectively. The heads of the embryos are always directed toward the cervix end of the uterus, and consequently point in a direction exactly opposite to that of the head of the mother. It was further found that the ventral embryo (I) is paired with the right-lateral (II) and that the dorsal (III) and left-lateral (IV) embryos are members of the other pair.<sup>6</sup> This relation was apparent, not only from the very close hereditary similarity existing between the two individuals of a pair, but also from certain foetal connections, notably the union of the amniotic canals of each pair in comparatively early stages.

The question now arises as to how such a striking relationship between the embryos has come into existence; and in seeking

<sup>6</sup> The terms 'right-lateral' and 'left-lateral' refer to the position of the embryos within the blastocyst, and not to the right and left sides respectively of the uterus. For example, the right-lateral embryo lies on the left side of the uterine cavity. Throughout the paper I use the Roman numerals (I to IV) to designate the embryos, so that the reader will find no difficulty in locating any embryo to which reference is made.

answer to this question we are at once confronted with the much more important problem of the origin of the multiple embryos from the single fertilized egg. In the paper cited above the position was taken that the embryos belonging to a pair were probably derived from one of the blastomeres of the two-celled stage, and that each embryo could therefore be looked upon as a lineal descendent of one of the blastomeres of the four-celled stage. In the present contribution the view is held that the four embryos do not owe their origin to a spontaneous blastotomy, but rather that they are the product of a form of agamogenesis belonging to the general category of budding.

It was considered then of the utmost importance to determine at just what point in the development of the armadillo blastocyst evidence of its quadruplicity first appeared. Consequently a sharp lookout was maintained in the study of all the early stages for signs of the first expression of polyembryony. The earliest observed evidence which could be interpreted as representing the beginning of multiple embryos comes in the formation of the mesothelium—not in the manner in which the elements of this layer arise, for localized centers of proliferation were not found, but in the early formation of the two large mesodermal vesicles through the fusion of several smaller ones. The development of two mesodermal vesicles would not in itself be so significant, as it might be merely an expression of a bilateral arrangement of mesoderm similar to that of many other vertebrate embryos, were it not for the fact that they hold a position corresponding exactly to the two primary ectodermal buds; that is, they lie on the sides of the vesicle which are directed towards the openings of the fallopian tubes. However, it may be that these two mesothelial vesicles have no general significance with reference to polyembryonic development, for it must be kept in mind that they have arisen by the fusion of numerous smaller vesicles, and later they in turn fuse to form a single vesicle.

Whatever may be the significance of the position of the two mesodermal vesicles, certain it is that the first indisputable evidence of the differentiation of the four embryos from the blasto-

cyst, appears in the formation of two diverticula from the ectodermal vesicle. In the preliminary paper these diverticula were termed the 'primary buds,' and I shall continue so to designate them. The buds appear on the opposite sides of the vesicle, and, with respect to the orientation of the blastocyst within the uterus, on the right and left sides, respectively, that is, on the sides of the vesicle that face the openings of the fallopian tubes. In accordance with the statement made above, the right primary bud faces the left fallopian tube opening, and similarly the left primary bud faces the right opening.

The primary buds do not develop for some time after the completion of the ectodermal vesicle, although their appearance is anticipated soon after this period by certain easily detectable changes in the walls of the vesicle. It will be recalled that immediately after the ectodermal sphere has become transformed into a vesicle, that portion of the wall of the vesicle which is turned toward the free pole of the blastocyst is of a relatively uniform thickness (fig. 51). Very shortly thereafter one can detect a tendency in this region of the wall to become less thick (figs. 55-58). The thinning out may be due in part to an increase in size of the vesicle by the accumulation of fluid within its cavity, but undoubtedly in the main it is brought about through the shifting of cells from here to the lateral portions of the wall, for these show an increase in thickness (fig. 59).

The shifting of the cells from the pole of the vesicle results in the formation of a thickened zone adjoining the thin or endothelial-like portion of the ectodermal vesicle (figs. 59, 61). The zone is not uniformly thick, but is thickest at the two regions corresponding respectively to the right and left sides of the vesicle. One can therefore correctly speak of these thickened areas as lateral plates.

The primary buds arise from these lateral plates, and appear as two broad, blunt processes protruding from the sides of the ectodermal vesicle (fig. 21). Each bud involves the greater portion of the side of the vesicle, covering an arc of approximately 80 degrees on the circumference. These points can be made out in specimen No. 247, which will now be described.

In the preserved condition, the chorionic vesicle measured 0.722 mm., from right to left, at the base, and the ectodermal vesicle 0.443 mm. in the same plane; while in the antero-posterior plane it measured 0.866 mm., and the vesicle 0.312 mm. The ectodermal vesicle is, therefore, approximately a third wider in the right-left plane than in the antero-posterior plane, and this difference is due to the presence of the primary buds. In

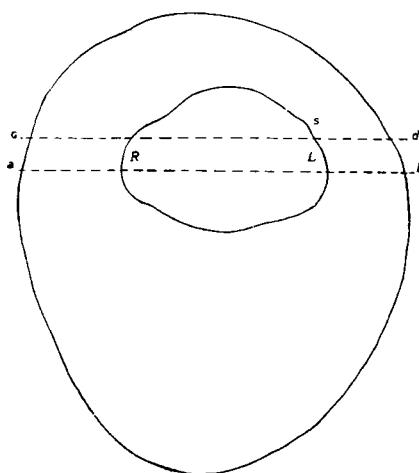


Fig. 1 Outline reconstruction of specimen No. 247. This shows the well formed 'primary buds,' situated on the right and left hand sides of the vesicle. The broken line shows the plane of the two sections illustrated in plate 6, figures 60, 61.  $\times 95$ .

preparing the specimen for microscopical study the sections were cut so as to pass parallel to the long axis of the ectodermal vesicle (fig. 1).

In the median sections of the series the primary buds are instantly recognized on the right and left sides of the ectodermal vesicle. They appear as outgrowths from the vesicle, and their cavities (fig. 22, *R* and *L*) are seen to be extensions of the common amniotic cavity. The wall of each bud is three or four cells thick, while the roof of the vesicle has thinned out to a single layer in thickness. Furthermore, the cells in the walls

of the buds are undergoing a rapid increase, as is evident from the numerous mitotic figures seen here.

On the lower side of the ectodermal vesicle the wall is composed of a single, thin layer of cells, and below this is the layer of mesoderm. It will be recognized that these two layers together constitute the true amnion, out of which the amnion of each embryo will eventually arise.

The mesoderm of the amnion is a portion of the mesothelium, the origin of which was described in a preceding section. The extraembryonic body cavity occupies the entire space lying between the amnion and the mucosa, and only traces (fig. 22) of the partition of the two original mesodermal vesicles remain to tell the history of this large cavity.

The outer (upper) surface of the chorionic vesicle consists of entoderm. This unique condition has been brought about through the disappearance of the chorionic ectoderm (*hinfälliges Ectoderm* of Fernandez '09), which has sloughed off. Its disappearance may occur prior to this period (fig. 20), or it may persist even until the four embryonic rudiments are established (fig. 23). The ectoderm breaks off just beyond the point where the entoderm unites to its inner surface (fig. 22, *x*), and thus exposes the entire outer surface of the entoderm to the cavity of the uterus. This condition persists throughout the entire period of gestation, in a manner to be described later.

Prior to the formation of the primary buds, the ectoderm and the adjacent entoderm remain distinct from each other, as seen in such specimens as those shown in figures 58 and 59; but, upon the development of the buds, these two layers are brought into intimate contact, which at certain points amounts practically to a fusion between the two layers. These points are situated just above the central area of each primary bud, and therefore mark the general region of the primordia of the future embryos.

No important changes in the extraembryonic mesoderm appear to be taking place at this time. It retains its epithelial-like character and no cell proliferations are found. A few mesoder-

mal cells are found just beyond the outer margin of the embryonic ectoderm, in the space lying between the entoderm and the extraembryonic mesoderm, but such cells are undoubtedly given off from the marginal cells of the ectoderm.

#### ORIGIN OF THE SECONDARY BUDS

The formation of the secondary buds immediately follows the establishment of the primary diverticula, and three or four specimens in the collection show the main steps in the process. However, it will be necessary to secure a closer series through this period of development before a detailed account can be given of the origin of the secondary buds. Each primary bud gives rise to two secondary buds, and consequently there are four secondary diverticula. Each secondary bud carries the rudiment or primordium of an embryo. The first step leading to the development of the secondary diverticula consists in the formation of two thickenings in the wall of each primary bud. One of these areas lies at the tip of the bud, while the other appears slightly to the left (as viewed from above) of the tip. The secondary buds then arise from these areas as blind diverticula, which extend down along the inner surface of the yolk-sac entoderm. In specimen No. 247 the beginning of the secondary buds can be seen in the left-hand primary bud (fig. 1). At the point marked *s* is seen a slight protrusion which will form secondary bud No. III.

The secondary buds soon become recognizable in surface views of living specimens, and appear as four blunt processes from the sides of the ectodermal vesicle. Upon the upper surface of each bud an embryonic rudiment appears, in the form of a white, opaque spot. It is somewhat difficult to make out the exact limits of the different parts of the ectodermal structures, owing to the fact that the entoderm (and the chorionic ectoderm, if still present) tend to obscure the view. This difficulty was obviated by making an outline reconstruction from the series of sections of the blastocyst.

The reconstruction of the specimen, which in point of development comes closest to No. 247 (fig. 1), is represented in figure 2, in which the outer, circular line marks the margin of the

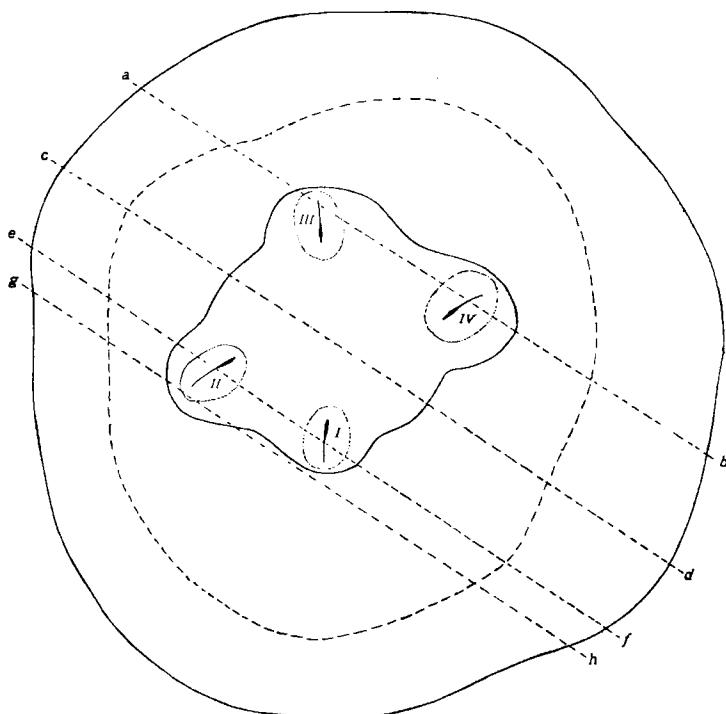


Fig. 2 Outline reconstruction of specimen No. 290. The outer circular line marks the margin of the blastocyst; the broken line indicates the upper limit of the Träger; and the diamond-shaped central figure is the outline of the ectodermal vesicle. The four oval figures lying within the ectodermal vesicle are the embryonic areas. The parallel broken lines represent the planes of the sections shown in plate 7.  $\times 63$ .

blastocyst, the broken line the upper limit of the Träger, the central irregular line the limits of the ectodermal vesicle.

Our attention must naturally be centered on the ectodermal structure. Its general outline is much like that of a diamond, in which the points are occupied by the secondary buds, or em-

bryos. The 'right-lateral' (II) and 'left-lateral' (IV) embryos lie at the acute angles of the diamond, while the 'dorsal' (III) and 'ventral' (I) embryos occupy the obtuse angles. The same condition prevails in two specimens but slightly older than this one; hence we may conclude that it is the normal arrangement of the secondary buds.

It is certain from the evidence that Embryos I and II are the product of the right-hand primary bud, while Embryos III and IV have come from the left-hand primary bud. It might appear from an examination of figure 2 that the secondary buds at II and IV had alone come from the two primary buds of such a specimen as No. 247 (fig. 1), while those at III and I had arisen *de novo* from the dorsal and ventral sides, respectively, of the ectodermal vesicle. But the entire genesis of these secondary buds argues against such an interpretation, as will become clear after a complete account of their development has been given. Nevertheless, it is an interesting fact, and one I believe not to be without significance, that the secondary buds II and IV occupy positions on the periphery similar to those of the two primary buds. Furthermore, it is probably correct to regard Embryos I and III as having arisen as outgrowths from the left sides (assuming that the observer is situated in the center of the free surface of the blastocyst) of the right and left primary buds, respectively.

The oval figure in each bud indicates the area over which the ectoderm and entoderm have come into intimate contact or have fused, while the solid line within each oval area shows the position and extent of the primitive groove. The head of the future embryo will, in each case, be directed towards the center of the vesicle.

The plane of the sections is indicated by the broken parallel lines, which also show the position of the four sections from which the photographs of plate 7 were made.

The section passing through the plane *e* to *f* will be taken for detailed account of the different parts of the chorionic vesicle. The outer membrane of the chorionic ectoderm (fig. 23) shows signs of breaking away. It has usually disappeared before this

time; thus exposing the entoderm to the uterine cavity. The entoderm is connected at the margin to a thickened portion of the chorionic ectoderm, which is recognized as the Träger (*Tr.*).

The mesothelium which lines the extraembryonic cavity (*E. E. B. C.*), has undergone no important changes; but there has been organized just beneath it a distinct Träger epithelium (*Tr. Ep.*), which in its relation to the uterine mucosa is particularly clear in the photograph (fig. 64).

The most important changes involve the ectodermal vesicle, and chief among these is the one affecting the organization of the embryonic primordia. On account of the inversion of germ layers, the lower side of each embryonic primordium is uppermost in the figure, that is, the lower side of the future embryo will be on the outside of the ectodermal vesicle. The section under discussion shows the rudiments of Embryos I and II, which are cut across at a slightly oblique angle (fig. 2). Each embryonic spot is characterized by (1) a fusion between the ectoderm and the entoderm, and (2) by a rather shallow depression, or primitive groove, which is situated on the lower or inner side of the embryonic ectoderm (fig. 23, *P. G.*). Between the two embryos the entoderm is entirely free from that portion of the ectoderm which joins the adjacent sides of the two embryonic rudiments. The cavity lying below the embryos is the bay of the original right-hand primary bud, and is a portion of the general amniotic cavity. The thin lower wall of this cavity is, therefore, the ectodermal layer, which, together with the adjacent mesodermal epithelium, constitutes the true amnion (fig. 23, *Am.*).

The primitive groove of each embryo measured about 0.095 mm., and is rather shallow throughout the greater part of its length. There is, however, a pit-like depression at one point which undoubtedly corresponds to a primitive pit. This is especially distinct in Embryo I, in which it lies about 0.032 mm. from the anterior end of the groove (fig. 24, *P. P.*).

Beneath the primitive groove there is the typically early primitive streak region, from which cells are being proliferated laterally to form the true embryonic mesoderm (fig. 24, *E. Mes.*). Throughout the extent of the primitive streak there is no

evidence that the underlying entoderm takes part in this proliferation of the mesodermal cells, but directly anterior to the primitive streak the entoderm is found to be actively dividing to form a group of cells, which can be traced throughout successive stages to mesodermal tissue. Undoubtedly this center of proliferation corresponds to the protochordal plate of Hubrecht (fig. 25, *P. Pl.*).

The conditions which we have here described for the pair of Embryos I and II also hold for the other pair, or Embryos III and IV. The relation of the various parts is identical in the two pairs. The only point in which they do differ is the fact that the two secondary buds III and IV are more widely separated than I and II, and hence the ectodermal vesicle appears much wider (*cf.* fig. 62 with fig. 64).

Following the sections through from the anterior limits of either pair of embryos towards the center of the vesicle, two important changes are noticeable: First, the entoderm becomes entirely separated and distinct from the ectoderm; that is, these two layers have never become fused; and second, the roof of the ectodermal vesicle thins out to a single layer, while to either side its wall remains from two to three cells thick (fig. 63). These lateral thickenings are found prior to the appearance of the embryonic rudiments, when the roof of the vesicle undergoes the general reduction in thickness.

Toward the posterior ends of the embryos the posterior grooves fade out, completely disappearing before the sections which cut the tip of the primary buds are reached (fig. 26). In the case of Embryos III and IV the posterior ends of the embryos extend well back into the primary buds, which are seen to be sharply separated from each other, especially in the last three or four sections which cut the ectodermal vesicle (fig. 26).

The embryonic entoderm or gut-entoderm of each embryo is differentiated from the primary yolk-sac entoderm. Apparently any region of the yolk-sac where the embryonic buds happen to impinge against its inner surface will differentiate into gut-entoderm.

The conditions which we have just recorded may be further chronicled by a brief account of specimen No. 175, which is the

next oldest blastocyst in the collection. In the entire blastocyst the primary buds with their accompanying embryonic rudiments stood out clearer than in the preceding specimen (No. 290), mainly because the chorionic ectoderm had already sloughed off from the upper side of the vesicle, thus giving a better view of the ectodermal vesicle.

The general arrangement of the secondary buds is identical in these two specimens (*cf.* fig. 2 and 28), but No. 175 is distinctly further developed. This becomes especially evident in a detailed study of the sections. In the section which passes through the place *a* to *b*, figure 29, bud IV is seen to be well defined, and the accompanying embryonic rudiment extends down into the tip of the bud, showing a well defined primitive groove (fig. 66). Anteriorly the groove becomes very pronounced, due in a large measure to the elevation of the medullary folds (fig. 67).

In the central region of the ectodermal vesicle (fig. 68) the lateral walls have become distinctly thinner, and will soon reach a state in which the entire wall, exclusive of the embryonic portions, will become but a single layer thick. When this condition is once attained the ectodermal vesicle undergoes no further growth or expansion, but remains a small inconspicuous structure (common amniotic vesicle) to which the embryos remain connected by means of amniotic canals or tubes. These canals are developed through the extension of the secondary buds, which rapidly push outward and then downward along the under side of the entoderm, carrying with them the embryonic rudiments. At this point we are concerned with the beginning only of the tubes, and this can be seen not only in figure 66, but also in figure 69, especially in the case of Embryo II.

It remains to say a word about the cavity lying just beneath the Träger epithelium in blastocyst No. 175. This cavity extends throughout the greater part of the left side of the chorionic vesicle (fig. 27, *Cav.*). If these figures are compared with text figure 1 of Fernandez ('09) it will be found that there is much similarity between them as regards this cavity. Fernandez designates it the Träger cavity (or Ectoplacentarplatten-

höhle) in *Mulita*; but in the Texas armadillo the enlarged space is clearly an artifact. One can easily observe in the sections that it has been produced by lifting up the Träger epithelium from the subjacent emaciated mucosa, probably through the action of the fixing and hardening reagents. It has not been observed in any other specimen, either older or younger, and this leads one to suspect that it is likewise an artifact in the blastocyst of *Mulita*, especially as it does not appear in the older vesicles of this animal.

In plate 9 is shown a series of five sections from a chorionic vesicle presenting a further advance in the development of the secondary buds and their accompanying embryos. The specimen is one of the finest in my collection, not only because of its excellent state of preservation, but also for the reason that it remained turgid while undergoing fixation, and thus gives us a picture in the sections which most closely resembles that of the living vesicle.

Figure 3 is an outline reconstruction of this series, and shows that the buds have made considerable progress. Buds II and IV are still larger than I and III, but this inequality gradually grows less and less as the buds extend outward and downward beneath the entoderm.

In the section passing through plane *e* to *f* of figure 3, the general relation of the various parts is well shown. The large extraembryonic cavity, lined with mesoderm, is conspicuous. Above this, and separated by the thin amnion, is the amniotic cavity of the ectodermal vesicle (fig. 72). The section passes a little to the left of the center of the left-lateral bud, which appears in section as a prolongation of the left side of the ectodermal vesicle. The outer covering of the chorionic vesicle is the entoderm, the chorionic ectoderm having already disappeared. The point at which it has broken off close to the base of the vesicle is clearly seen in this and the other photographs.

The other sections illustrated in the plate present special parts of the several embryos. Thus figures 70 and 74 show transverse sections of Embryo III and Embryo I, respectively. In each case the primitive groove is distinct. In figure 71 the

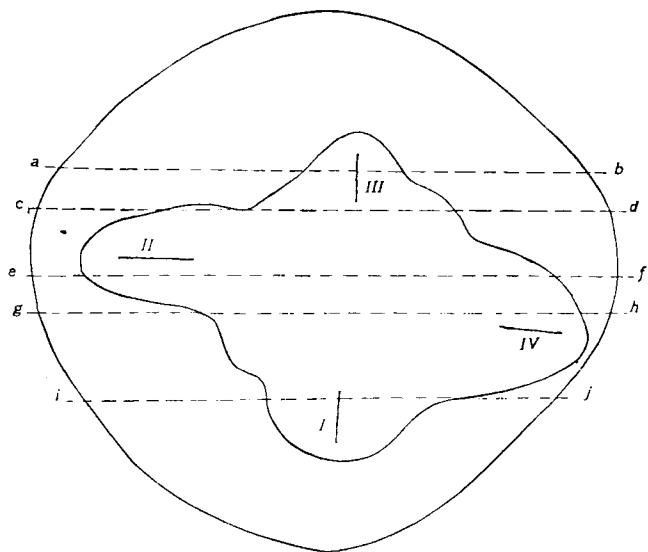


Fig. 3 Outline reconstruction of the left half of specimen no. 257. The five sections from this specimen are illustrated in plate 9.  $\times 83$ .

section passes through line *c* to *d* of figure 3, and thus cuts across the angle lying between Embryos II and III. Finally figure 73 passes a little to the left of the center of Embryo IV, and shows the structure of that embryo.

At the tips of the buds the cells are in active division (fig. 30), indicating that the extension of the buds, during their early existence, is to be accounted for in this way. Their subsequent extension is due to other factors, which will be considered in the next sections.

#### EXTENSION OF THE SECONDARY BUDS AND ORGANIZATION OF THE EMBRYOS

The further extension of the secondary buds from the sides of the ectodermal vesicle very rapidly follows such conditions as we have described in connection with specimen No. 257. A rapid growth or increase in size of the vesicle also follows or accompanies this extension of the buds. Each bud grows down along the inner side of the wall of the vesicle, between the entoderm and the meso-

thelium, eventually establishing a placental connection with the extending Träger, thus forming a sort of 'belly-stalk' through which the placental blood vessels run, and into which the rudimentary allantois later extends. In their growth from the vesicle the buds do not pass out as four distinct rays from a common center, the common amniotic vesicle, but extend out in pairs, the individuals of each pair retaining a common connection with the ectodermal vesicle. The paired condition is but a further expression of the same relation which was noted in connection with the account of the origin of the secondary buds.

The earliest phase of this condition is very clearly brought out in one of the specimens of the series. This specimen has almost completely collapsed and is inclined to the left. Hence Embryos I and II are in part folded beneath the wall of the blastocyst, making their study difficult.

In plate 10 are shown photographs of three sections which pass through Embryos III and IV at different levels. Figure 75 is taken across the common bay of the two embryos, about half way between the common amniotic vesicle (remains of the old ectodermal vesicle) and the point of departure of the two secondary buds. The chorionic ectoderm has disappeared and consequently the entoderm is the uppermost layer. It is entirely distinct from the embryonic ectoderm.

Figure 76 represents a section which passes through the point where the secondary buds arise and diverge. The bud on the left contains Embryo IV, and its width is almost twice that of its paired mate on the right, or number III.<sup>7</sup> The difference between the two buds exists throughout their entire length. Thus in the section cutting the middle of the buds (fig. 77) the difference in size is particularly striking. Each embryo consists of the following parts: (1) the entoderm, which is in contact with the primitive streak mesoderm; (2) the primitive streak mesoderm, which is being proliferated from the ectoderm; (3)

<sup>7</sup> In the preliminary paper (figs. 8 and 9) these embryonic buds were incorrectly labeled. This was due to the fact that the sections had inadvertently been reversed in mounting them on the slide, a fact not discovered until after plate 10 of the present paper had been made up.

the thick embryonic ectoderm, curved upward on each side; (4) the thin amniotic ectoderm above; and finally, (5) the mesoderm of the false amniotic or extraembryonic cavity.

Briefly stated then, each embryo consists of a tube-like out-growth from the ectodermal vesicle, with which it retains a

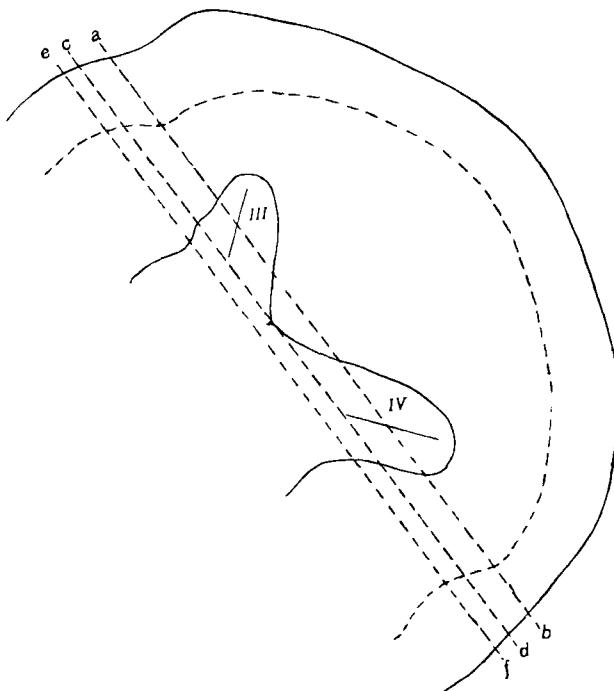


Fig. 4 Outline reconstruction of the left half of specimen No. 170. Three sections from this specimen are shown in plate 10, figures 75 to 77.  $\times 62$ .

connection in the form of the proximal part of the secondary bud. As a result of the manner in which the secondary buds arise from the primary ones, this connection is common to two embryos, which always constitutes a pair.

Aside from the differentiation or organization of the embryos, the final stages in the extension of the secondary buds presents very little of special interest. We may therefore refer to them briefly. First of all, it should be stated that following a stage

such as we have just considered the vesicle grows rapidly in size, quickly filling up the entire lumen of the uterus. The first noticeable change in growth affects the free or distal portion of the wall, and the chorionic vesicle soon becomes bulb shaped (fig. 5).

The expansion and growth of the wall of the blastocyst gradually carries distally the common amniotic vesicle, which by this time has ceased to grow. As a result, the embryonic rudiments are gradually separated from the amniotic vesicle, and their organic connections with the vesicle are drawn out into small tube-like structures, the amniotic connecting canals. These canals lie against the under or inner surface of the entoderm, and each consists of an inner layer of ectoderm, surrounded by a layer of mesoderm.

In plate 11 are shown five sections, taken at different levels, from a specimen in which the amniotic vesicle was just beginning to be drawn away from the embryonic rudiments, through the expansion of the wall. The sections are not quite transverse to the axes of the four embryos.

The section represented in figure 80 cuts the tip of the chorionic vesicle and passes through the lower portion of the common amniotic vesicle. The anterior parts of three of the embryos are seen in the section. These are the dorsal, right-lateral, and ventral embryos. The position of the other embryo, or the left-lateral, is indicated by the evagination at the left side of the vesicle.

In figure 81, which is three sections further down, the same relation with reference to the embryonic rudiments still exists, but the section passes through the extreme lower limit of the amniotic vesicle, and the right-lateral embryo becomes entirely separated from the others.

In figure 82, which is five sections lower down on the chorionic vesicle than figure 81, the dorsal and right-lateral embryos are both free from any connections with the amniotic vesicle. In section each embryo appears as a section of a tube. The chief interest in this section lies in the condition of the ventral and left-lateral embryonic rudiments. Only the anterior tips of

these two embryos are shown, and they lie each at the end of a common bay by means of which their amniotic cavities are placed in communication with the cavity of the common amniotic vesicle. This condition is of the greatest significance, since it indicates the common origin of the pair of embryonic tubes from the left-hand primary bud.

Figure 83 shows all four of the embryonic rudiments in section lying on the sides of the wall of the blastocyst, and facing on the inner side of the large extraembryonic cavity.

Figure 84 is taken about half way between the posterior ends of the embryonic tubes and the base of the chorionic vesicle. Here the wall of the blastocyst is composed of the typical structures, entoderm on the outside and mesoderm within. The latter is rapidly becoming vasculated, especially in the regions lying directly posterior to the embryonic tubes.

Soon after the stage just referred to, the chorionic vesicle begins a very rapid expansion, and, in doing so, first assumes a bulb-like shape (fig. 5). The vesicle is united to the mucosa by an annular zone of thickened trophoblastic ectoderm, the so-called Träger (fig. 23), and from the edge of this the yolk sac entoderm extends upwards to form the outer layer of the chorionic wall, the chorionic ectoderm having long since disappeared. The inner layer of the chorion everywhere consists of mesoderm.

The embryonic portion of the vesicle consists of the relatively small, common amniotic vesicle (fig. 5, *C A. V.*), from the right and left sides of which spring a pair of small connecting canals. Each canal places the cavity of the amniotic vesicle in direct communication with the amniotic cavity of the embryo. It will be recognized that these canals are the elongated proximal parts of the original 'secondary buds', and that the embryonic rudiments are the distal portions of such buds. This is the reason why the canals spring in pairs from the vesicle. However, there is considerable variation in the relation of the pair of canals to the vesicle. The one presented in figure 5 is rather unusual, in that the amniotic vesicle is greatly elongated, instead of spherical, and the two canals of a pair arise very close together from the end of the vesicle. The usual condition shows

the pair of canals united at a very short distance from the vesicle, and thus entering it as a single short tube. Variations from this are seen in those cases in which the union takes place further and further away from the amniotic vesicle. In one of the most extreme cases observed the two canals on one side were united for a distance of about three millimeters from the vesicle, or more than half way between the anterior end of the embryo and the amniotic vesicle.

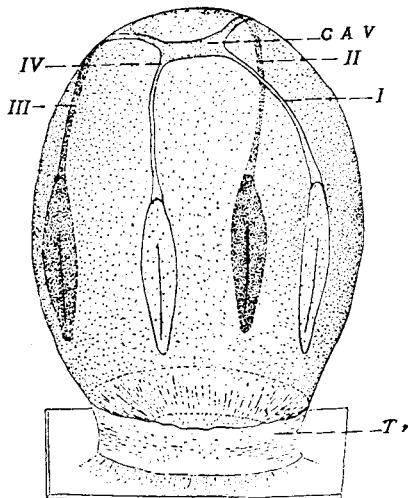


Fig. 5 A free hand drawing of specimen No. 276, in which the embryonic rudiments are well started. Each embryo is connected with the common amniotic vesicle (C.A.V.) by means of a slender tube-like canal. In the living condition the vesicle measured about 5 mm. high by 3 mm. at the widest point.  $\times 11$ .

The important point is that in all of these cases the paired condition of the embryos is unmistakably clear, and I know of no other way to account for this except to assume that it rests upon the manner in which the pair of secondary buds arises from the primary buds. If the secondary buds start soon after the primary buds are formed, than a condition similar to that seen in figure 5 might readily result; but if the primary bud has made considerable progress in its extension from the vesicle before the secondary buds arose from it, then we should expect

to find a common canal for the two embryos developing out of the proximal part of the primary bud.

Each embryonic rudiment is a slipper-shaped structure lying at the terminus of a canal (fig. 5), and is in a relatively late primitive streak stage. At the anterior end of the embryo the medullary plate is well formed, although as yet the medullary folds have not become elevated. Posteriorly the embryo ends as an irregular, blunt process of mesodermal tissue.

Fig. 78 shows a transverse section of one of the embryonic tubes from specimen No. 276. The section passes through the embryo at the level of the extreme anterior tip of the primitive streak, and hence cuts the thickened medullary plate, which curves upward and inward to become the thin ectodermal layer of the amnion. Beneath the medullary plate, and between it and the entoderm, are seen the scattered mesodermal cells which have been proliferated from the primitive streak.

Lateral to the embryo is a loose mass of mesodermal cells which lie between the entoderm and epithelial-like mesoderm of the extraembryonic cavity. In the whole condition of the chorionic vesicle the mesoderm was seen to fringe each side of the embryonic tube, extending throughout the entire length of the embryo proper. It already shows a rudimentary net-work of blood-vessels, which represents the beginning of the area vasculosa. I have not worked out the detailed history of the vascular area, but undoubtedly it arises in each embryo in a manner similar to that in the typical mammalian ovum from which but a single embryo develops.

The embryonic mesoderm is thickest at the posterior end of the embryo, where it gives rise to a series of enlargements which extend for some little distance behind the extreme posterior tip of the embryonic tube. This is the portion of the mesoderm into which the umbilical vessels and the rudimentary diverticulum of the allantois later extend; that is, it forms the basis for the belly-stalk.

The further development of each embryo is very similar to that of the ordinary mammal, and therefore calls for a brief description only. The reader is referred to an earlier paper in

which is found an account of the late stages of development (Newman and Patterson '10).

The differentiation of the embryo within the tube is soon made evident by the elevation of the neural folds to form the neural tube, and by the cutting off of paired mesodermic somites. As these changes in the embryo are progressing, the tube-like amnion is rapidly undergoing modification. First, it develops a finger-like prolongation which extends back beyond the posterior limit of the embryo. Then there follows a rapid expansion of that portion of the amniotic tube which is occupied by the embryo, the anterior portion of the tube remaining small, and, together with the common amniotic vesicle, is destined to degenerate and disappear.

During the expansion the amnion at first assumes a cigar-like shape, and the amnia of the four embryos soon come to fill the entire cavity of the chorionic vesicle, each embryo and its membranes occupying one quadrant of the vesicle. In the mean time, each embryo has become constricted from the extra-embryonic parts by the development of the characteristic head, tail, and lateral folds, and retains its connections with the chorion by means of a typical umbilicus. It thus floats quite freely within the amniotic fluid.

During all of these changes the paired condition of the embryos is perfectly distinct, and, in the final stages of development, expresses itself in the arrangement of the embryos into right-hand and left-hand pairs, as is evidenced by the umbilical connections with the placenta (fig. 79).

#### IMPLANTATION OF THE OVUM AND PLACENTATION

The more general features of the late phases of placentation have been presented in another paper, but most of the material for the study of the early development of the placenta has but recently been secured. The nature of this paper does not call for a detailed account of this process, and even though it did, it would not be possible to write it in full at this time, since one or two of the critical stages have not yet been obtained.

Particularly is this true of the changes which immediately follow the attachment of the blastocyst. It is therefore proposed briefly to describe some of those changes involved in the early history of implantation and placentation which are essential to a clear understanding of the embryology of the armadillo. This account has been deferred until now, because it will be somewhat easier to follow after the description of the development has been read.

A statement as to the manner in which the embryonic vesicle migrates along the horizontal groove of the uterus and eventually attaches itself has been given above. The principal facts are as follows: After the blastocyst has reached the placental area, which lies at the extreme anterior tip of the fundus, it adheres to the mucosa, apparently for some time before the actual placentation or intimate union with the uterine membrane is consummated. Four clear and two somewhat doubtful cases of early adhesion between the ovum and the mucosa have been observed. In every instance the ovum freed itself either immediately or very shortly after the application of the fixing fluid, thus indicating that the implantation proper had not really taken place. Fortunately, these vesicles were studied in salt solution under the binocular microscope before fixation and it was possible to make out several important points. It was observed that the ovum always comes to rest upon the uterine surface in such a way that the embryonic spot or germinal area is turned toward the mucosa. Hence, the foetal contribution to the early placenta arises from that portion of the trophoblast which overlies the inner cell-mass; that is, from the so-called Rauber's layer.

It is to this region of the trophoblast then that one must look to detect the first indications of placentation. In five of the blastocysts to which we have just called attention no evidence of importance in this connection was observable, but in the sixth unmistakable indications of early placentation are present. In this specimen Rauber's layer is seen to have undergone important modifications. Several of the cells are in mitosis, and all of the cells of this region have lost their original attenuated

appearance, and now show a decided increase in thickness (fig. 11). There is also a distinct tendency for some of the cell walls to disappear, thus transforming Rauber's layer into a syncytium. Furthermore, the surface of Rauber's layer presents a 'fuzzy' appearance, the outer wall of some of the cells actually being broken as though ruptures had resulted when the blastocyst was freed from its insecure moorings to the mucosa.

That all of these facts are evidence of the beginning of implantation is clearly indicated by the act that the trophoblastic cells which lie beyond Rauber's area are still unchanged, and show the mosaic-like arrangement of polygonal cells so characteristic of all of the free blastocysts. Unfortunately, there is here a slight break in the series, so that we are not able to follow up this clew through the obviously critical period of implantation. We are therefore obliged to pass directly to an account of the modifications which are occurring both in the mucosa epithelium and in the wall of the blastocyst in a vesicle which has already become firmly anchored to the maternal tissue.

Blastocyst No. 316 represents the youngest firmly attached stage that has been secured. A series of sections from this specimen is shown in plate 2. In the living vesicle it could be seen that, in addition to the small area which had established an intimate union with the mucosa, almost the entire left side was lying in contact with the uterine wall, and that as a result the trophoblastic cells here had greatly increased in thickness (fig. 16). Whatever may be the nature of the stimulus which causes the trophoblastic cells to react whenever brought into close relation with the uterine epithelium, it is certain that the influence is not confined to Rauber's layer, but any portion of the trophoblast, upon coming in contact with the mucosa, will respond. It does not necessarily follow that such thickened trophoblast establishes eventually a fusion or placental union with the uterine wall, although a study of the stages more advanced than this one, suggests that some of it may so unite. However, it should be pointed out that only in rare instances does a blastocyst become attached in a manner such that an

extensive area of the trophoblast is brought into close contact with the mucosa. Whenever the blastocyst happens to become anchored at the bottom of a furrow or on the side of a fold, as in the case of No. 316, it follows that a larger area of contact is possible than when its union is established on the top of a fold or on a perfectly smooth surface.

Although there is an extensive area of contact in the specimen under discussion, yet the place of fusion is indeed small, and does not occupy a space any greater than that previously covered by the Rauber's layer. The chorionic vesicle rests upon a mass of trophoblastic tissue which is in the form of a concave disc, with the concave side directed upwards, and with the upper margin of the disc passing insensibly into the free trophoblastic portion of the vesicle (fig. 31).

The disc or primitive placenta is comparable to the attachment mass of rodents, and has been termed the Träger. In the armadillo the Träger arises through the formation of the syncytium in Rauber's portion of the trophoblast, followed by a fusion of this syncytium with the surface layer of the mucosa. The fused mass thus forms a bridge across which the embryonic nuclei can pass from the syncytium into the maternal tissues, portions of which are soon destroyed by these invading nuclei, doubtless as a result of their phagocytic or histolytic action.

In specimen No. 316 the fusion is firmly established and several embryonic nuclei have already penetrated well into the mucosa epithelium (fig. 31, *Em. N.*). Several other nuclei are on the point of passing into the mucosa. Evidences of the histolytic properties of these foreign nuclei are everywhere present in the maternal portion of the fused region, and the nutritive substances which result from the breaking down of the maternal tissues must serve as an embryotrophe. The embryotrophic phase of placentation must last throughout a relatively long period of the early development, because neither the maternal nor foetal circulation is established in the placenta until the embryonic rudiments are well formed.

In the next stage, which is but slightly older than No. 316, a somewhat larger number of nuclei have passed over into the uterine wall. They have almost completely destroyed the epithelium, and have also affected the sub-epithelial layers (fig. 17). The syncytium and adjacent thickened trophoblast together form a layer which is coextensive with the germinal spot, that is, they extend to the margin of the entoderm. In addition there is found on the left wall, and somewhat removed from the rest of the thickened extraembryonal ectoderm, a distinct knot of cells which must have resulted from the contact of a very small spot of the trophoblast with the mucosa (fig. 17, *K*).

The next important change is a spreading of the base of the vesicle. Instead of having but a small area of contact or point of union, as in specimen Nos. 316 and 332 (figs. 31 and 17), the blastocyst soon acquires a base, often greater in width than that of the entire free portion of the vesicle (fig. 18). As a result, the wall of the chorion, upon approaching the mucosa, instead of curving inward, now slopes gradually outward, and merges into the uterine tissue with which it has established a very intimate union. The base forms an annular zone of thickened trophoblast, which in width extends from the margin of the entoderm to the surface of the uterine wall. In its more distal part the annular zone consists of thickened cells (two or three cells thick), but its proximal part is much thicker and one finds here a tendency to form a syncytium, continuous with the syncytial-like mass lying directly beneath the chorionic vesicle.

In the latter region are found numerous embryonic nuclei, which have destroyed the mucous epithelium, the connective-tissue stroma, and even portions of the uterine glands. These nuclei may be divided into two groups, one of which occupies a superficial position; that is, its elements border on the extraembryonic cavity and apparently are inactive in so far as the destruction of the mucosa is concerned; and the other constitutes the deeper lying nuclei which are instrumental in dissolving the mucosa. At first this distinction is not very evident, (figs. 18 and 32), but as development progresses the protoplasm surrounding the surface nuclei becomes more and more dense

(fig. 33), and there is gradually evolved a distinct epithelial layer (figs. 33, 34, 64). Following the suggestion of Fernandez ('09) in his paper on *Mulita*, we may designate it the 'Träger Epithelium,' on contrast to the Träger zone, or thickened annular portion of the trophoblast which forms the base of the attached chorionic vesicle. The Träger epithelium, together with the layer of extraembryonic mesoderm which directly overlies it, forms the lower wall of the chorionic vesicle.

In the meantime the deeper-seated nuclei continue to migrate farther into the uterine tissue, which, as already stated, soon becomes destroyed. During these changes the upper portion of the syncytium begins to degenerate, as is evidenced by the appearance of numerous vacuoles (figs. 34, 35). The vacuoles flow together and eventually produce a shallow cavity which lies just beneath the now well-organized Träger epithelium. The final condition in the development of this 'sub-Träger cavity' is well illustrated in the series of photographs in plate 7. All of these show the condition just referred to, but figure 64 is particularly clear. Here the sub-Träger cavity is sharply defined above by the Träger epithelium, but on the lower side it is connected with numerous spaces which lie between the remains of the partly dissociated mucosa.

Occasionally during the process of fixation the Träger epithelium becomes pushed up into the extraembryonic cavity, carrying before it the layer of mesoderm. Under such conditions it appears as though the Träger epithelium was normally arched (figs. 66-69). I am inclined to believe that a similar artificial condition in the chorionic vesicle of *Mulita* has lead Fernandez ('09) to conclude that the Träger epithelium is normally pushed up into the extraembryonic cavity, and that consequently the cavity lying just below it is to be regarded as a true Träger cavity. In view of the fact that Fernandez had only a few scattered stages at command, it was easy for him to be mislead into drawing this conclusion. But the evidence which I have just presented from a study of the development of this cavity makes it doubtful whether it should be regarded as a Träger cavity, in the sense in which that term is used in rodents. For that

reason I have preferred to call it by the name of sub-Träger cavity.

A further examination of the figures on plate 7 will show that at this stage of development the blastocyst is held to the mucosa by the Träger zone alone, so that in order to free it from the uterine wall it is merely necessary to cut this band of tissue at a short distance beyond the edge of the vesicle.

The transition from the Träger stage or primary placenta to that of the secondary placenta, or a condition in which distinct villi are present, is very gradual. The first villi arise from that portion of the chorionic wall which corresponds to the Träger zone; that is at the attached margin of the chorionic vesicle. From here they successively appear further and further toward the central area of the Träger epithelium, until its entire surface becomes studded with them. However, the villi in the central region do not become so long or so highly developed as those that are situated towards its peripheral parts.

Each villus starts as a thickening in the Träger epithelium, and soon becomes a mass of cells protruding from the epithelial surface. At first it is flat or disc-shaped and seems to serve as an adhesive pad, but later it elongates into a true Träger cord, which may become very much branched. These cords later become invaded by a stroma-like mesenchyme, developed from the mesodermal epithelium which directly overlies the Träger epithelium.<sup>8</sup>

In order to understand the changes which take place in the upper free portion of the chorionic wall it is necessary to recall that all of the trophoblast lying above the margin of the entoderm sooner or later sloughs off, leaving only a fragment-like base, which in section can often be seen protruding from the side of the vesicle at a short distance above the uterine surface. This fragment is clearly shown in all of the figures of plate 9.

The portion of the trophoblast which thus breaks away corresponds to the chorionic ectoderm in the vesicle of other mam-

<sup>8</sup> For a more detailed account, and for figures of the structure of the villi, see Newman and Patterson, '10.

mals, but in the blastocyst of the armadillo its loss results in bringing the yolk-sac entoderm to the outer surface. From now on the increase in size of the blastocyst is evidently brought about through the growth of this entodermal portion of the wall of the ovum, as can be determined by comparing successive stages. But after the vesicle has attained a diameter of from 4 to 5 mm. (fig. 5) the entodermal portion does not expand to any great extent, and the chorionic vesicle owes its further growth to the extension of the Träger or placental portion of the chorionic wall.

As the Träger area increases in extent the yolk-sac is carried farther and farther toward the cervix end of the uterus, and in the advanced stages of gestation it forms a cap at the tip of the cervix end of the chorionic vesicle. However, it becomes partly covered by an overgrowth of Träger tissue, which arises at the boundary line between the yolk-sac and the Träger, and extends posteriorly as a free margin which later becomes fused with the wall of the cervix. In vesicles which contain 30 to 35 mm. embryos, the yolk-sac portion of the chorion protrudes through the thickened ring-like, placental overgrowth as a clear transparent membrane.

If a chorionic vesicle from which the chorionic ectoderm has already disappeared be examined, it is found that the entoderm is attached to the upper edge of a mass of Träger tissue which lies just inside the basal fragment of trophoblast (figs. 70-74). In tracing back the origin of this mass one finds that it arises as a thickening on the inner surface of the Träger zone, just below the margin of the entoderm. It can be recognized in very early stages as a small mass of actively dividing cells which lie in the angle formed by the Träger zone and the potential Träger epithelium (fig. 55, on left). The thickening gradually increases in volume (figs. 33, 34, *m*), and apparently involves the entire inner surface of the Träger zone, and thus comes to form at its crest a fusion with the margin of the entoderm (fig. 23, on right). At the same time the outer layer of cells of the Träger zone becomes split off from the mass (fig. 23, on left), and thus forms the basal fragment when the chorionic ectoderm breaks away.

The mass of Träger tissue constitutes the material upon which the further growth of the chorionic wall depends, and after the 4 to 5 mm. stage is reached it rapidly extends upward, becomes thinner, and carries before it the cap of the yolk-sac entoderm, in the manner already described. It also forms the basis for the formation of the villi which appear upon this region of the chorionic wall. At first the villi have a general distribution over the surface of the modified Träger zone, but in blastocysts which have attained a diameter of 25 to 30 mm., the long branched villi become restricted to four distinct areas or patches. These patches are situated just below the boundary line between the Träger and the yolk-sac, and each villous area lies opposite the point at which an umbilical cord arises from the inner surface of the chorionic wall. The four villous areas give rise to the four placental discs of later stages, and, corresponding to the position of the embryos, are arranged into two pairs. In the final stages of gestation the two discs belonging to a pair become closely associated together, thus forming two large double-discoidal placenta, which occupy respectively the right and left sides of the chorion.

In the formation of the placenta, as well as in the general development of the blastocyst of the armadillo, there are many opportunities for comparison with the developmental stages of other mammals, but such comparisons can be more safely drawn after we have had a chance to work out a detailed history of placentation.

#### GENERAL DISCUSSION

##### *1. Theories of polyembryony*

A great many different views have been expressed in explanation of polyembryonic development. Most of these are pure conjectures, and as such hold no place in any serious attempt at a scientific treatment of the subject. Exceptions are made here to those theories only which seem to hold a grain of truth and which have gained a certain number of adherents.

*a. Theory of polyovular follicles.* An attempt has been made to account for polyembryony on the basis of polyovular follicles.

Since it was known that the mammalian ovary occasionally possesses such follicles, it was natural to suppose that this fact might furnish a clew to the problem of polyembryony. In fact, Rosner ('01) not only made this claim, but he also attempted to prove his point by a study of the ovaries of the South American variety of the very animal with which this paper deals, namely, the nine-banded armadillo. Rosner studied a single pair of ovaries from a pregnant female sent him by von Jhering. He found fifty-two polyovular follicles, as follows: one with seven ova, one with five, two with four, seven with three, and eleven with two. Rosner believes that the condition of multiple embryos in the armadillo was to be explained by assuming that four young, adjacent follicles fused together, and that the four ova thus brought within a single cavity were later ovulated and fertilized, and held together in such a way as to produce eventually the quadruplex foetal structure characteristic of the armadillo pregnancy.

Aside from the fact that only two out of the fifty-two polyovular follicles observed by Rosner possessed the requisite number of ova to account for the four embryos of each armadillo litter, it has since been abundantly proved (Cuenot '03, and Newman and Patterson '10) that the pair of ovaries studied by Rosner was quite exceptional. Further, sufficient literature has accumulated to show that the phenomenon of polyovular follicles occurs in several widely separated species of mammals, and consequently can have nothing to do with polyembryonic development.

Schrön ('63) seems to have been one of the first to record the occurrence of polyovular follicles. He observed them in the ovaries of the cat. Since then they have been reported in various Eutheria, as follows: in the human by Nagel ('88), Schottländer ('93), Stoeckel ('98), Rabl ('99), and Arnold ('12); in dogs by Waldeyer ('70), Wagener ('79), Bouins ('00) and Smyth ('08); in cats by Rabl ('99); in bats by Van Beneden ('80); in rabbits by Wagener ('79) and Honoré ('00); in the armadillo by Rosner ('01). In addition to these records on the Eutheria, O'Donoghue ('12) has very recently added the Marsupial, *Dasyurus viverrinus*.

These citations will suffice to indicate how widespread is the occurrence of polyovular follicles among mammals; but, although they have a distribution among widely separated forms, their occurrence in a given species seems to be rare. Thus O'Donoghue ('12) found them but twice in forty-five individuals of *Dasyurus*; and Schrön ('63) only twice in the ovaries of four hundred cats and but once in eighty dogs. The writer has examined in all probably more than fifty pairs of ovaries from the Texas armadillo without finding a single case.

In the light of these data it is impossible to associate the occurrence of polyovular follicles with the causation of polyembryonic development in mammals. The fact that Rosner has found a single armadillo with such follicles can have no greater importance than have the similar sporadic cases in certain other mammals. The significant fact is, rather, that in the ovaries of fifty individuals belonging to a species which reproduces by specific polyembryony alone not a single case of polyovular follicles was found.

It is well to emphasize the fact that the polyovular follicles do not lie at the basis of polyembryony, for to accept Rosner's theory would be equivalent to a denial altogether of the phenomenon of polyembryonic development in the Mammalia. A multiple gestation from ova which have accidentally become associated together during a part of the ovarian history, through the fusion of adjacent follicles, may have no more interest or significance than a similar gestation resulting from ova from uniovular follicles, but simultaneously ovulated, as occurs in many mammals that are normally multiparous. One cannot hope to throw much light on such fundamental biological problems as those of sex determination, the limits of hereditary control, and others, by the study of this type of development. It is only to those cases in which the several embryos of a multiple pregnancy have taken their origin from a single fertilized egg that we must look for facts with which to elucidate these problems.

It is not intended here to underestimate the biological importance of polyovular follicles and of multiple gestations other than those of polyembryony; but rather to point out that these

phenomena have an interest lying along a different line. In fact there is considerable evidence to indicate that polyovular follicles and such multiple gestations are to be correlated as cause and effect. This applies not only to cases of multiple pregnancies among forms that are normally uniparous, but also occasionally to cases in animals that are normally multiparous. According to Wilder ('04) von Franque ('98) was the first to start the discussion which has led up to the conclusion that polyovular follicles bearing two eggs might result in the origin of twins—not compound monsters or duplicate twins, but to fraternal twins, as Wilder points out, since the eggs must be fertilized by different spermatozoa.

The theory that polyovular follicles may account for 'fraternities' of this sort receives considerable support in the case of a dog reported by Smyth ('08). In 1906 he obtained a young setter pup from a litter of fourteen pups (four dogs and ten sluts) born to a Gordon setter. When the pup was ten months old she was spayed for reasons of convenience, and upon preparing the ovaries in sections it was discovered that not a few of the ripe follicles held double and triple ova. The ovaries from one of the other pups were also sectioned, and they too possessed polyovular follicles, one containing as high as seven ova. In 1907 one of the sluts from this same litter gave birth to nine pups, three dogs and six sluts. It is a great pity that the ovaries from this bitch were not studied, as well as those from her mother. But, even though the data are not as complete as might be desired, still they point unmistakably to the fact that a tendency to polyovular follicles was inherited in this family of dogs; and, furthermore, they suggest that the unusually large litters of the mother and her daughter might in part be accounted for on the basis of compound follicles.

It is a well-known fact that other normally multiparous animals sometimes show a tendency to bring forth very large litters. While it is of course possible that in such cases all of the ova belonging to a given litter may come from simple follicles, yet it is not improbable that some of them may come from polyovular follicles. There is but one way in which it would be possible to

settle this question satisfactorily, and that is to study histologically the ovaries of several generations in the family of some animal belonging to a species normally bearing a single young, but showing a tendency to multiple births. If polyovulated follicles were present in those individuals which had had multiple births and if the number of corpora present in the ovaries after a given pregnancy were found to be fewer than the number of young in the litter, it would be reasonably certain that the multiple gestations were the result of ova from a compound follicle. Some of the cases in cattle recently cited by Pearl ('12) would have made excellent material for such a test.

I have discussed the topic of polyovular follicles somewhat in detail in order to emphasize the necessity of keeping it entirely distinct from the subject of polyembryony, for not to do so will most certainly lead to great confusion. This applies not only to the problem of sex heredity, but also to other questions in heredity that are capable of elucidation through the study of polyembryonic development, particularly the one dealing with the limits of hereditary control. It is here essential, as I have already pointed out, that all of the individuals of a litter of embryos to be studied should have the same germinal constitution; but this condition would never be fulfilled in multiple gestations resulting from ova from a polyovular follicle, even if this could be proved beyond question, and even though all the ova came from a single mother cell in oogenesis; because in that event each egg must be fertilized by a different spermatozoon.

*b. Theory of blastotomy.* According to this theory each embryo is looked upon as the lineal descendant of one of the early blastomeres. In the case of two embryos arising from a single egg (identical twins) it has been supposed that each individual is the product of one of the blastomeres of the two-celled stage, while in the case of four embryos (armadillo quadruplets) it has been assumed that each embryo arises from one of the blastomeres of the four-celled stage. And so it has been argued for those cases in which even a greater number than four come from one egg.

The idea of an early spontaneous blastotomy lying at the basis of polyembryony has been very persistently urged by a number

of investigators, and in the earlier work on this armadillo the same view was held. This idea undoubtedly has its inception in, and has received most of its support from the results of certain experimental studies involving the mechanical or semi-mechanical separation of early blastomeres. For it has been demonstrated that an early isolated blastomere of the two-celled or four-celled stage of the eggs of the echinoderm (Driesch '92), medusa (Zoga '95-6), *Amphioxus* (Wilson '93), and teleost (Morgan '93) may develop into a complete but small larva, and even in the egg of the amphibian a blastomere of the two-celled stage, under certain conditions (Schultze '95, Morgan '95, and Herlitzka '97) may also develop into a complete organism. What then seemed more logical than to conclude that in the case of polyembryonic development the early blastomeres had in some way become displaced or isolated, and that each cell thus separated formed the center for a single individual. Moreover, this inference seemed all the more plausible in the light of certain studies on twins in the human species. Wilder ('04) in particular, in his extensive paper on duplicate twins and double monsters, advocates the theory that each member of a pair is the product of one blastomere.

The evidence that has been presented in the first part of this paper makes it certain that polyembryonic development in the armadillo cannot be explained on the basis of a spontaneous blastotomy, in the sense that each embryo is the lineal descendant of a single blastomere of the four-celled stage, and it causes one to view with some doubt the conclusions of this same nature that have been drawn by those who have worked on other polyembryonic forms. In this connection it should be kept in mind that, although an equipotentiality seems well established for the early blastomeres of the eggs of the echinoderms, medusa, *amphioxus*, teleost, and others, yet there are many forms in which a blastomere does not have the power to develop into a whole individual. Crampton ('96) on gasteropods, and Chabry ('87) and Conklin ('05, '06) on ascidians have conclusively demonstrated that a blastomere of the two-celled or four-celled stage

in these forms develops essentially in the same manner as though producing a part of the whole embryo.

It is not intended to deny that influences of a mechanical nature may not, in certain cases, lie at the basis of multiple-embryo formation. Any one advocating such a theory may bring to his support not only the facts of artificial blastotomy, but also those derived from experimental studies on later development, like those of the pioneer work of Haeckel ('69) on the blastulae of Crystallodes and of the more recent and well-known studies of Spemann ('01, '03) on the triton egg. This rather simple mechanical or semi-mechanical explanation might hold in the sporadic cases of polyembryony, like those of duplicate twins and double monsters, but what evidence have we that blastotomy operates in the case of specific polyembryony in higher forms? As yet we know very little about the details of the early development in such cases. It is a significant fact that such evidence as we do possess does not support the theory, and it is certainly true that these studies on the armadillo—a form in which we have a most striking case of specific polyembryony—have not revealed any evidence which tends to support the blastotomy theory.<sup>9</sup> On the contrary, the evidence points unmistakably to a different explanation, namely, that a type of budding lies at the basis of polyembryony in this form.

c. *The theory of budding.* The process of budding is a very common method of reproduction among organisms. In plants it is practically universal, and in animals it is frequently met with, especially among the lower forms. In many cases asexual reproduction by budding occurs late in the life cycle, as for example among coelenterates. In such forms as the common *Hydra* it is customary to regard the organism as an adult when budding begins. But the appearance of budding is by no means confined to adult organisms, or even to late stages of development, for it may appear very early in the life cycle.

<sup>9</sup> The term 'spontaneous blastotomy' has been used by Bugnion and Marchel to describe the process of polyembryony in the parasitic Hymenoptera, but not in the sense that each embryo can be traced to a single blastomere. Brandes ('98) has suggested the term 'Germmogonie' in lieu of polyembryony.

In the earthworm, Kleinenberg ('79) has described a gemelli-parous development occurring in the gastrula stage and initiated by a sort of fission or budding. In the Cyclostomatous Bryzoa (Harmer '93, Robertson '03) a primary embryo, prior to the formation of germ layers, buds off a large number of secondary embryos which differentiate into larvae. In the parasitic Hymenoptera (Marchal '04, and Silvestri '06) the differentiation of the embryos occurs relatively still earlier and consists in the dissociation of the egg into embryonic masses, which vary in number according to the species, and which later form larvae. In the light of artificial blastotomy it is possible, theoretically, to have the process of dissociation carried still further forward into the cleavage stages—even to the two-celled stage; but so far as the writer knows, the occurrence of blastotomy as early as the two- or four-celled stage has never been observed as a natural phenomenon in development.

From among the several forms having polyembryonic development, one can select a series in which embryonic fission or budding is carried farther and farther toward the adult stage, and as Marchal has observed, one can pass insensibly from these cases of budding in the egg to the more frequent and well-known phenomenon in which the budding occurs after the individual has already come from the egg, as, for example, in the coelenterates, Orthonectida, Dicyemida, platyhelminthes and tunicates. We may ask then, below what point in the developmental cycle must one cease to speak of asexual reproduction as budding, and refer to it as polyembryony? Evidently a sharp line cannot be drawn between the two.

It is best to regard polyembryony as a precocious type of budding; and this, perhaps, only in the sense that it occurs early in the embryonic life, and without the implication that it has pushed forward in the life cycle or superseded a budding which in the ancestral forms occurred at a late period of development. This would seem to be the case at least in the Polyzoa, in which the embryonic budding is followed in the sessile larval stage by the typical budding to produce the colony.

In the armadillo the facts of development which we have presented in the descriptive part of this paper are fully in accord with the theory of budding. We have seen that the early phases of differentiation are similar to those of other mammals in which the ovum produces but one embryo. Prior to the appearance of the embryos, the ectoderm, the entoderm, and the exocoelomic mesoderm are differentiated, and later all three of these layers are concerned in the formation of the embryonic buds. The initial step in budding apparently occurs in the embryonic ectoderm, but the entodermal layer is soon involved in the process. It is therefore entirely correct to say that the seat of budding in the armadillo is to be found in the blastoderm, that is, the blastoderm in the budding organ. It may be possible to extend this same conception to accidental or sporadic cases of polyembryony occurring in the lower forms which lay yolk-laden eggs.

The most important point brought out in this study is the fact that polyembryonic development in the armadillo can be interpreted as a type of budding; and, while to show that polyembryony is a budding process does not solve the question as to the determining cause of the division of the blastoderm, yet it is a distinct step toward the solution of that important problem.

It is perhaps premature to attempt an explanation of the ultimate cause of polyembryony. We first need a comprehensive study of each of the forms in which it occurs. Such investigations, followed by well-directed experiments, may yield results that will reveal at least some of the factors which control polyembryonic development. At present only a few suggestions need be made; and, first, we may briefly consider the ideas that have been expressed by some of those who have worked on the subject.

Harmer ('93), in his excellent paper on embryonic fission in Bryozoa, points out several interesting comparisons that can be drawn between the process of multiple-embryo formation in *Crisia* and budding in many other organisms. He calls attention to the fact that, in at least some of these forms, embryonic fission is connected with the deviation from the normal type of

segmentation of the egg. Furthermore, he points out that the early blastomeres of the egg of *Crisia* are separated from each other by follicle-tissue, and that they are surrounded by a rich nutritive material, which is obtained through the protoplasmic strands connecting the several units of the colony with the ovi-cell. He believes that the production of numerous larvae from the primary embryo in *Crisia* is a process comparable to artificial blastotomy in *Echinus* eggs, as shown by the experiments of Driesch ('92). His general conclusion is clearly stated in the closing paragraph of his paper. He says:

The cases already quoted may be taken as showing that some of the abnormalities in the development of *Crisia* may be due to the nutritive conditions in which the development takes place. Just as the presence of food-yolk within the egg modifies the character of the segmentation and the formation of the layers, so the presence of copious stores of nutrient material in the maternal tissues outside the egg may also effect the early developmental processes. Thus the large number of relatively large larvae which develop from the minute egg of a *Crisia* could not be produced if the egg were not supplied with nutriment from outside itself. While some of the irregularity in the segmentation of the egg may be due to this cause, the extreme independence of the blastomeres at an early stage may be connected with the acquirement by the embryo of a habit of forming buds in the embryonic condition.<sup>10</sup>

Marchal ('04) has expressed somewhat similar views, as may be gathered from the following quotation.<sup>11</sup>

As to the determining cause of the division of the germ, Marchal thinks that it is from the sudden surrounding with more dilute liquids in the interior of the nourishing mass and in a concomitant modification of the osmotic exchanges in the interior of the cellules. One sees, in fact, with Encyrtus that polyembryony reaches its greatest intensity at the moment when the larvae of the *Hyponomeuta* commences to feed (in the early days of April), and for the *Polygnotus* at the period when the young larva of the Hessian fly engorges itself with sap. Now, the production of the rapid changes bringing about osmotic pressure constitutes precisely the procedure employed to bring about the separation of the blastomeres and their evolution into several distinct individuals, as has been shown by the experiments already mentioned of Loeb and Bataillon.

<sup>10</sup> Loc. cit., p. 236.

<sup>11</sup> From Howard's ('06, p. 816) clear translation of Bugnion's ('06) review of Marchel's ('04) paper.

Moreover, in connection with his study on *Polygnotus*, Mar-chal observed that the polygerm is moved back and forth in the digestive tract as a result of contractions of the wall of the host. He believes that this movement is analogous to the shaking of *Echinus* eggs, and has a similar influence upon the division of the germ.

In the preliminary paper (Patterson '12) similar views were given, but expressed in a somewhat different way. It was stated that in all of the well known cases of polyembryony the cleavage of the egg is of the 'indeterminate' type, so that it was impossible to trace out a 'cell-lineage' for any particular embryo. It was also stated that the primary embryo or polygerm led a sort of parasitic existence, and that as a consequence it was surrounded an abundance of nutritive substances.

The cleavage of the mammalian egg is generally regarded as belonging to the indeterminate type, and, although the cleavage stages of the armadillo have yet to be studied, still we have no reasons for believing that they will be found to differ from those of other mammals; and if we may judge from the conditions of the earliest stages of the blastocyst that have been examined, there is no evidence to show that the early blastomeres have been separated by foreign nutritive substances. The development of the embryonic vesicle until the germ layers are differentiated can be compared to that of certain other mammals. However, it is a significant fact that at the close of the period of germ layer formation the embryotrophic phase of placentation, which is particularly striking in the armadillo, becomes well established. It may be that the nutritive substances produced by the action of the embryonic nuclei upon the maternal tissue furnish the stimulus which excites the blastoderm to bud off the embryonic tubes, just as the engorged sap of the Hessian fly is suggested by Mar-chal to be the determining cause of the division of the germ of *Encyrtus*. If this point be well taken, it is evidently not necessary to assume that the time of stimulation to polyembryonic development dates back to the early cleavage stages.

In this connection it might be well to call attention to another suggestion that has been made. It is generally believed that in

coelenterates, ascidians, and Polyzoa the germ cells antedate the formation of the buds in which they are found; and Montgomery ('06) has gone so far as to suggest that perhaps in all cases the products of asexual reproduction contain germ cells. He suggests that "If this were so, it might then be the case that the incapacity of any part of the body of an animal to reproduce asexually or even to regenerate, would be due to the absence of germ cells in it." It may be possible to explain in this way the appearance of rudimentary embryos that have been observed in both species of armadillo, or even, indeed, to account for the so-called asexual larvae in *Litomastix*. Such abortive attempts to produce normal organisms may be the result of a failure on the part of a bud to receive germ cells.

## *2. Origin of polyembryony in the Dasypodidae*

One of the interesting problems which presents itself in connection with this study pertains to the question of the origin of specific polyembryony among the Dasypodidae. The question becomes all the more interesting, from the standpoint of the physiology of reproduction, in view of the fact that the uterus of the armadillo is of the simplex type. The same type of uterus in other mammals is adapted to the function of receiving and nourishing a single ovum, although occasionally it may receive and nourish two or more ova at the same time, in which event a multiple birth results ("fraternal twins," "triplets," etc.). In the armadillo the uterus also receives a single ovum at a time, but instead of but one embryo arising from the egg, polyembryonic development comes on and increases the number of offspring to several individuals. Here we have a clear case of adaptation, in which the productivity of the ovum of a uniparous mammal is increased several fold.

Two quite distinct problems are presented. One of these is the most fundamental question of the whole problem of polyembryony, namely: What are the causal factors underlying the formation of two or more individuals from a single egg? The other problem concerns the phylogeny of specific polyembryony in

the group of armadillos. It must be stated frankly that both of these questions are wholly unanswered. But a few suggestions are given in the hope that they may point the way to their solution. In the remaining part of this section we shall discuss the question of phylogeny as the other problem was discussed in the preceding section.

So far as I am aware, the only armadillos in which specific polyembryony is definitely known to occur are the North and South American varieties of *T. novemcincta*, and *T. hybrida*, which inhabit the southern part of the South American continent. Judging from the description of Fernandez ('09) on comparatively late stages of *T. hybrida*, the two species agree very closely in many details of development; but there are evidently important differences, one of which relates to the number of offspring in a litter. In *T. novemcincta* a litter consists typically of four individuals, while the number in *T. hybrida* varies from seven to twelve, with a strong tendency to produce eight or nine.

In *T. novemcincta*, out of 219 pregnant females that have been studied carefully, 176 had embryonic vesicles far enough advanced to permit a determination of the number of embryos, and of these all but four, or about 98 per cent, possessed four embryos each. Of the four exceptions, three had five-embryo sets and one a two-embryo set.

There is some doubt in the latter case, since the embryos were born in the laboratory and therefore an opportunity to study them in utero was not offered. But all of the five-embryo sets were carefully studied and the relations of the different parts of the blastocyst were determined. In each case two members of the litter (either on the right or on the left side) showed the normal paired condition, while the other three presented an asymmetrical arrangement, one of the primary placental discs being about twice the usual size and bearing the umbilical cords of two embryos.

This arrangement of the members of a five-embryo set is significant, in that it suggests that the two embryos arose in the normal fashion from one of the primary buds, and that the three embryos of the opposite side have come from the other primary

bud, either directly, through the formation of three secondary buds from it, or through a further division of one of the two secondary buds, after they had been formed in the normal way.

It is to be regretted that the rare case of a two-embryo set, referred to above, was not studied before birth occurred, as it would doubtless have been found that the embryos occupied the right and left sides of the chorionic vesicle, thus indicating that each primary bud had been directly transformed into a single individual. In that event, we should have had very strong evidence in support of our contention that specific polyembryony in the Dasypodidae began by the formation of a set of twins, perhaps at first as sporadic cases of gemelliparous development such as probably occurs in the production of duplicate twins in the human species.

However that may be, I have recently discovered certain evidence in the early development of the Texas armadillo which strongly supports this view concerning the origin of specific polyembryony in the Dasypodidae. It was pointed out in an earlier section that when the secondary buds first appear, Nos. II and IV apparently arise from the tips of the primary buds, as though they were merely prolongations of these buds; while each of the other secondary buds evidently arises slightly to one side of tip of a primary bud. That is to say, that Embryo I always arises to the left of its paired mate No. II, and likewise Embryo III to the left of IV (fig. 2). This may be expressed in another way by saying that buds I and III are outgrowths from the primary buds, and that consequently they follow chronologically the development of buds II and IV. The evidence upon which this interpretation is based is to be seen in several of the young blastocysts.

It has been pointed out elsewhere that the first sign of a secondary bud appears on the right side of the left-hand primary bud in blastocyst No. 247 (fig. 1). The other embryonic bud is the result of a prolongation of the extreme tip of the primary diverticulum. In figures 2, 3, 4, and 28 is seen further evidence of this same difference in the size of the two members of a pair of embryos, and it is also evident in the sections (figs. 67, 77).

Furthermore, it was observed in at least three living specimens, so that there can be no doubt but that during the early history of the embryonic buds, Nos. II and IV are more highly developed than their mates I and III. And what else can this mean than that phylogenetically the former pair should precede the latter.

This obvious difference in size does not last indefinitely, and indeed is no longer discernable after the embryonic rudiments have become slipper-shaped (fig. 5).

That primary buds may also be precursors of secondary buds in *Mulita* is, I believe, to be inferred from the work of Fernandez ('09) on that form. Unfortunately, he has but a few scattered stages at his command, and therefore was not able to give us a full history of the development of the embryonic rudiments. His specimen 46, which is the one next to his youngest stage (corresponding to Specimen 233), already has embryonic rudiments fully as well developed as those in Specimen No. 226 (plate 11). Nevertheless, in speaking of the relation which exists between the ectoderm of the common amnion and that of the embryonic diverticula in this specimen, he makes the following significant statement, "Der Cervix uteri zu, d.h. über dem Anfang der Medullarplatte, ist das Amnion keines Tieres geschlossen, as steht vielmehr durch eine sehr weite Öffnung mit dem Amnion eines oder mehrerer Nachbartiere in vollkommener Kommunikation."<sup>12</sup> Again in the same paragraph he says, "Das Amnion eines Einzelembryo kann sich auch direkt in diese Blase öffnen, ohne vorher mit den Amnia anderer Embryonen in Verbindung getreten zu sein."

If we pass from these two statements to an examination of his figures 1 and 2 (plate 17), which are photographs of vesicles showing well-formed embryos, we shall find further evidence of this same nature. The specimen shown in figure 1 is from a vesicle containing 11 embryos, six of which appear in the photograph. On account of the advanced stage of development, the evidence that two embryos have come from a common diverticulum must be sought in the relation of the amniotic canals to the common

<sup>12</sup> Loc. cit., p. 314.

amniotic vesicle. Curiously enough it is found in two or three instances that the canals of two embryos unite and enter the vesicle as a single tube, and in one case at least three canals so unite. His figure 2 presents a still more striking case. It contains 9 embryos, and the embryo lying at the top of the figure, and slightly to the right of the center, sends its canal directly to the vesicle, while the canals from the four embryos on the right enter the vesicle very close together, two of them by a common tube. Likewise, the canals from the four embryos lying on the left enter the vesicle at a common point. Indeed, they apparently unite just before reaching the vesicle.

In view of the fact that we have shown that the union of two canals in *T. novemcineta* is a certain indication of their common origin from a primary bud, I believe we are justified in drawing a similar conclusion from the conditions to which we have just called attention in *T. hybrida*. And I venture to predict that when Fernandez shall have secured intermediate stages, he will be able to confirm this conclusion.<sup>13</sup>

It does not follow, of course, that in *Mulita* only two primary buds will be found to appear, for while in *T. novencincta* the polyembryonic process in the ovum is extremely stable, as expressed by the constancy with which litters of quadruplets appear, in *T. hybrida*, on the contrary, variability characterizes this process. Hence, there are no good reasons why in this species, regions on the ectodermal vesicle which correspond to those unoccupied by the two primary buds in the vesicle of *novemcincta* might not give rise to new primary diverticula. If this be found to be the case it would in no wise nullify our conclusion regarding the origin of polyembryony among the armadillos; that is that it began in the ancestors by the formation of twins. Whether all of the species which might show such a primitive condition are

<sup>13</sup> After the above was written my attention was called to a report, in the Journal of the Royal Microscopical Society, June 1913, page 279, of Fernandez's communication at the Ninth International Congress of Zoologists. From the brief statement given it seems clear that Fernandez has observed exactly the same method of embryo formation in *Mulita* that I have described for *T. novemcineta*, that is, the embryonic primordia arise as diverticula. He states that the diverticula "*become the primordia of embryos, either directly or after division.*" (Italics my own).

now extinct is, of course, not known; but it can be stated that some of the living species, other than *T. novemcincta* and *T. hybrida*, are known to give birth sometimes to a single young, and at other times to two individuals.

### *3. Polyembryony and duplicate twins and double monsters*

Another interesting problem with which polyembryony is concerned is that of the origin of duplicate twins in the human species. There is a vast literature bearing on duplicate twins and various types of double monsters, and several different theories have been advanced to account for their production. The reader is referred to Wilder's ('04) extensive paper in which the more important references are cited, and in which the leading theories are discussed.

It is well at first to distinguish between the different kinds of twins, 'duplicate twins' and 'fraternal twins', understanding by the former those cases in which the two members of a pair are supposed to have come from a single egg, and by the latter those supposed to be the product of two distinct eggs. This distinction is by no means an artificial one, but is based upon a considerable amount of data. It is supported not only by the general physical appearance of the members of a pair, but also by the intra-uterine relationships of the two members. In fraternal twins the two individuals do not resemble each other any more closely than do the several individuals of a litter belonging to a normally multiparous animal, and the intra-uterine relationship of two chorions indicate that their origin is from two distinct eggs. In duplicate twins the individuals are enclosed within a single chorion, and their close resemblance to each other is often so striking that it has gained for them the name of 'identical twins.' Furthermore, duplicate twins are invariably of the same sex, while fraternal twins may be of the same or of different sex.

Wilder points out that this fundamental principle upon which the distinction between duplicate and fraternal twins is based, also holds in multiple births involving more than two individuals, and that it can be extended to include cases of duplicate twins and similar combinations in other forms.

As to the origin of duplicate twins, Wilder advocates the blastotomy theory, believing that it is the result of a total separation of the first two blastomeres of the single egg. In case the blastomeres fail to separate completely, symmetrical double monsters (*dislopagi*) result. In this connection he says, "The double monsters of which we have authentic record are sufficiently numerous and diverse to represent every stage from that of the otherwise normal individual with a doubling of certain of the median parts, to that of two complete duplicate twins with a slight connection between them."<sup>14</sup> Finally, he believes that unequal duplicate monsters (*autosite* and *parasite*) are the result of a secondary fusion (due to the great contiguity) of two embryos which were at first duplicate twins.

The contention that duplicate twins and double monsters arise from a single egg is undoubtedly sound, but the conclusion that their origin is the result of a complete or a partial separation of the first two blastomeres, is, I believe, open to question. However, in the absence of any study in the early stages of these sporadic cases of polyembryony, and in view of the results from experiments on artificial blastotomy, this conclusion seemed both natural and logical. In the study of the embryology of the armadillo we have a most excellent opportunity to put the blastotomy theory to test; for here is a mammal with a simplex type of uterus, and one which habitually reproduces by polyembryony. I am therefore bold enough to suggest that the conclusions which I have drawn concerning the origin of the embryos in this mammal may also apply to cases of duplicate twins and double monsters in the human species. And I am encouraged to make this suggestion because of the recent discoveries in the human ovum (e. g. the Bryce and Teacher ovum, '08), in which the condition of the ectodermal vesicle is shown to be such as to require no great stretch of the imagination to picture how diverticula might arise from it, and thus initiate the development of two or more embryos. Nor is there any greater mental strain in accounting for the origin of composite monsters in this way than is required in the hypothet-

<sup>14</sup> Loc. cit., p. 462.

ical juggling of blastomeres to account for the various relationships and positions assumed between the components of these monsters.

#### *4. Polyembryony and sex*

One of the obvious biological bearings that the study of polyembryonic development has revealed concerns heredity, including the heredity of sex. The illuminating studies of McClung ('03), Stevens ('06), Wilson ('05-'12), Morgan ('09) and many others, including the recent excellent paper on *Ancyracanthus* by Mulsow ('12), have shown that in a large number of animal forms the heredity of sex is in some way bound up with certain co-called sex chromosomes, and that, as a consequence, the sex of a given individual is irrevocably fixed at the time of fertilization, or in the case of an unfertilized or parthenogenetic egg, not later than the time the egg starts to develop.

It is in this connection that polyembryonic development furnishes a very strong confirmation of the modern cytological view of sex heredity. For in every authentic case of polyembryony among dioecious species all of the individuals arising from one egg are invariably of the same sex; that is they are either all males or all females, never mixed. The most logical conclusion that can be drawn from these facts is that the sex character is stamped upon the egg prior to the origin of the several individuals to which it gives rise. That the sex of the egg is determined as early as the time at which development begins, seems certain in the case of parasitic hymenoptera. It will be recalled that Silvestri ('06) has shown in *Litomastix* (and he thinks that is is almost certainly true in *Ageniaspis*) that the fertilized egg produces females only, while the unfertilized egg gives rise to males only, exactly as in the well known case of the bee. Here fertilization or the lack thereof determines the sex of the offspring, and, no matter how many individuals the egg may produce by polyembryonic development—and in *Litomastix* the astonishingly large number of about 3000 may develop—they are all of the same sex.<sup>15</sup>

<sup>15</sup> Except, of course, the so-called asexual larvae—the origin and development of each needs further study.

It might be argued that the identity of sex among the several individuals of a polyembryonic litter is the result of similarity of environment. But here again the facts of fertilization in the development of *Litomastix* completely disproves the idea that external influences are in any way causal factors in sex determination, at least in this parasitic insect. Furthermore, in the case of the development of the armadillo the four embryonic primordia early become separated from each other, each embryo becoming enclosed within its own amniotic envelope, and in a great measure acquiring its own individual environment. All of this takes place long before the sexual organs develop, indeed, long before the so-called 'hermaphroditic stage' of the embryo appears. If external factors play any role in sex determination, it is difficult to understand, under the conditions obtaining in the armadillo, why litters containing both male and female individuals are never produced.

The study of polyembryonic development in *Litomastix* also calls to mind another important fact, viz., that polyembryony has nothing to do with the actual determination of the sex of the egg. This conclusion becomes evident if one considers the different sexual conditions which exist in the several species exhibiting polyembryony. It occurs in the typical dioecious species, like the armadillo, in which the fertilized egg produces all females or all males, it occurs in the parasitic hymenoptera, in which the fertilized egg produces females, and the unfertilized egg males; and finally, it occurs in hermaphroditic forms, like the earth worm, and also (probably) certain cyclostomatous Bryozoa.<sup>16</sup>

Polyembryonic development may obtain, therefore, no matter whether the egg be fertilized or not, or whether it is destined to bring forth a progeny of unisexual or one of bisexual individuals. Let us repeat, then, that polyembryonic development is not to be considered as a causal factor in sex determination. The facts of polyembryonic development adds, rather strong corroborative evidence to that of cytology, namely, that the sex potentiality of the egg is fixed at a very early stage of development—doubtless in all ordinary cases, at the time of fertilization.

<sup>16</sup> Robertson ('03) states that *Crisia geniculata* and *C. cornuta* (or *edwardsiana*, according to her later identification, '10) are probably monoecious.

## SUMMARY

The main facts brought out in the descriptive part of the paper may be summarized as follows:

1. The arrangement of the folds of the uterine mucosa is such that there is formed a distinct cross-shaped area of comparatively smooth mucosa at the tip of the fundus. The center of the cross is the attachment zone for the embryonic vesicle, and its right or left arm, depending on which ovary functions, forms the pathway along which the vesicle travels from the Fallopian tube to the point of attachment (fig. 21, pp. 561-563).
2. The armadillos breed during October and the early part of November. A large majority of the old females are pregnant before October 15, but the second year females may continue to breed for some time after this period. The young are born in March and April, but an occasional litter may appear in February. The period of gestation is estimated at one hundred and forty days (p. 564).
3. Except in very rare instances, young females born in March or April do not breed during the succeeding fall (p. 564).
4. A 'period of quiescence' of the blastocyst was discovered. This period lasts for at least three weeks, and is probably similar to the quiescent period of the blastocyst of the deer described by Bischoff (pp. 564-565).
5. The right and left ovaries function with equal frequency (p. 567).
6. In no case has more than one ovum been found in the uterus (p. 567).
7. The youngest specimen obtained was a typical mammalian blastocyst, consisting of an outer trophoblastic layer of polygonal cells, and an inner cell-mass of embryonic cells (fig. 6, pp. 571-572).
8. The entoderm differentiates before implantation occurs, and arises through a migration of entodermal mother-cells from among the embryonic ectodermal cells of the inner cell-mass. These entodermal cells, migrate, either directly or after having undergone division, to the under surface of the mass, where they

first form a fenestrated structure which later is transformed into a continuous layer (figs. 8-14, 36-42, pp. 572-585).

9. After the entoderm becomes a continuous layer, it splits from the ectoderm, and its free margin passes beyond the limits of the ectodermal mass until the area covered by the entoderm equals an arc of about 80 degrees on the circumference of the blastocyst. The margin of the entoderm now unites with the trophoblastic wall. The remaining 280 degrees of trophoblastic wall never becomes lined with entoderm (figs. 15, 47, 48; p. 585).

10. The blastocyst becomes attached at the embryonic pole to the uterine wall. (figs. 16, 17, 43-46, 49, 50; pp. 585-587).

11. After the entoderm is split from the embryonic ectoderm, the latter rounds up into a spherical mass, which upon parting company with the trophoblast, pushes into the cavity of the vesicle and becomes included in the entodermal layer (figs. 15-17, 43-46, 48-50; pp. 587-590).

12. The ectodermal vesicle is formed by a vacuolization process, which results in disintegrating the core of the ectodermal sphere. When complete, the ectodermal vesicle consists of a single layered pole turned toward the Träger, and a uniformly thick pole, which faces the now inverted entoderm or yolk-sac (figs. 16, 17, 18, 43-46, 49-51; pp. 590-593).

13. During the process of inclusion of the ectodermal mass, there is created an extraembryonic cavity, which lies between the Träger and the endothelial-like pole of the ectodermal vesicle (figs. 17, 18, 49-51; pp. 592-593).

14. The extraembryonic mesoderm arises through cell proliferations from the ectodermal vesicle. These proliferations occur around the entire vesicle, at the angle formed by the vesicle and the entoderm, where the latter turns out to join the trophoblast. The mesoderm cells are given off in groups, which are quickly transformed into vesicular-like structures that fuse together to form a continuous lining or mesothelium for the extraembryonic cavity (figs. 19, 20, 55-60; pp. 593-595).

15. On the right and left sides of the ectodermal vesicle, the primary diverticula or buds appear from thickened areas that

have arisen through the shifting of cells from the thick pole of the vesicle (figs. 1, 21, 22, 59-61; pp. 598-603).

16. Soon after its origin, each primary diverticulum gives rise to two secondary diverticula or buds. One of these buds apparently is but an extension from the tip of the primary diverticulum, while the other takes its origin from the left-lateral portion of the tip of the diverticulum. The embryonic buds extend toward the Träger down along the inner side of the entodermal portion of the blastocyst wall as tube-like processes, which involve not only the thickened lateral plates of ectoderm, but also portions of the thin endothelial-like wall of the vesicle (figs. 23-29, 62-74; pp. 603-610).

17. The part of the ectodermal vesicle which remains after the embryonic tubes are given off becomes the common amniotic vesicle. It retains for some time connections with the embryonic tubes by means of the amniotic canals, which are differentiated from the proximal parts of the original diverticula. The characteristic paired condition of litters of *T. novemcincta* is the result of the method by which two secondary buds arise from each of the primary diverticula. The common amniotic vesicle eventually degenerates and disappears (figs. 5, 80-84; pp. 611-613).

18. Each embryo differentiates within the secondary diverticulum, deriving its ectoderm from a portion of the lateral plate which was carried down into the diverticulum, and its entoderm in loco from the primitive entodermal sac or yolk-sac. The embryonic mesoderm arises from a typical primitive streak region in each embryonic primordium (figs. 75-84; pp. 614-617).

19. The region of the so-called Rauber's layer forms the seat of attachment of the blastocyst. This region is soon transformed into a syncytium, from which the embryonic nuclei pass over into the mucosa, destroying it by their phagocytic or histolytic action (figs. 31-33; pp. 617-619).

20. The more superficially situated nuclei of the embryonic syncytium organize the Träger epithelium, which, together with the overlying mesothelial layer, forms the lower portion of the chorionic wall. The Träger proper, which forms a thickened annular zone about the base of the attached vesicle, gives rise

to the villi of the definitive placental discs. Villi also appear on the Träger epithelium but they remain more or less rudimentary (figs. 34, 35, 62-65; pp. 619-625).

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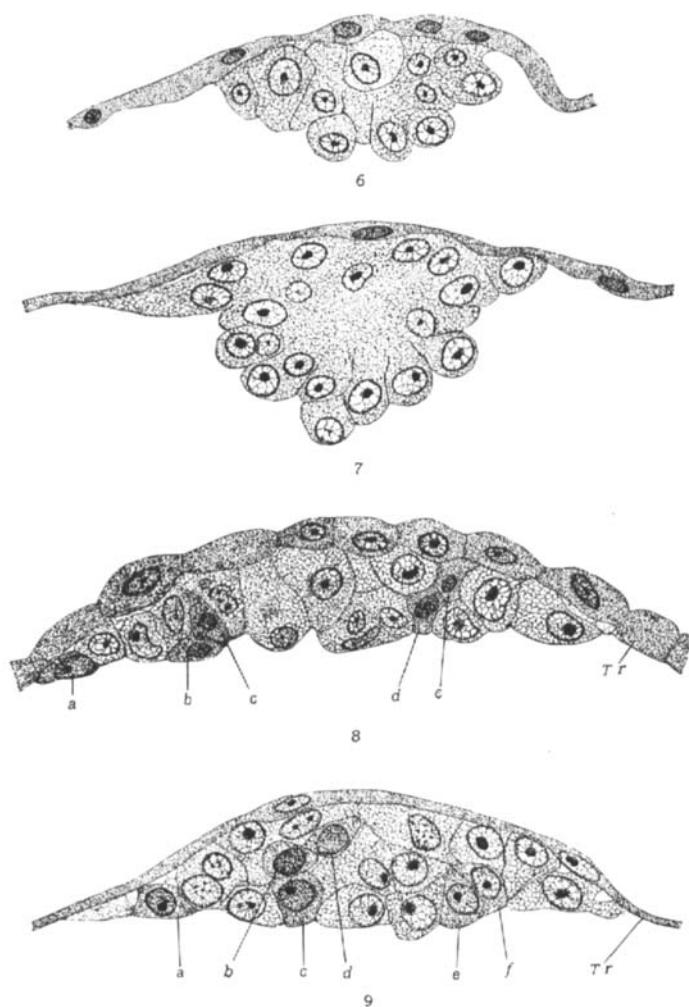


Fig. 6 Median section of the inner cell-mass of specimen No. 287.  $\times 913$ .

Fig. 7 Median section of the inner cell-mass of specimen No. 310.  $\times 913$ .

Fig. 8 Median section of the inner cell-mass of specimen No. 335. The letters *a* to *e* indicate the entodermal mother cells.  $\times 728$ .

Fig. 9 Median section of inner cell-mass of blastocyst No. 244.  $\times 728$ .

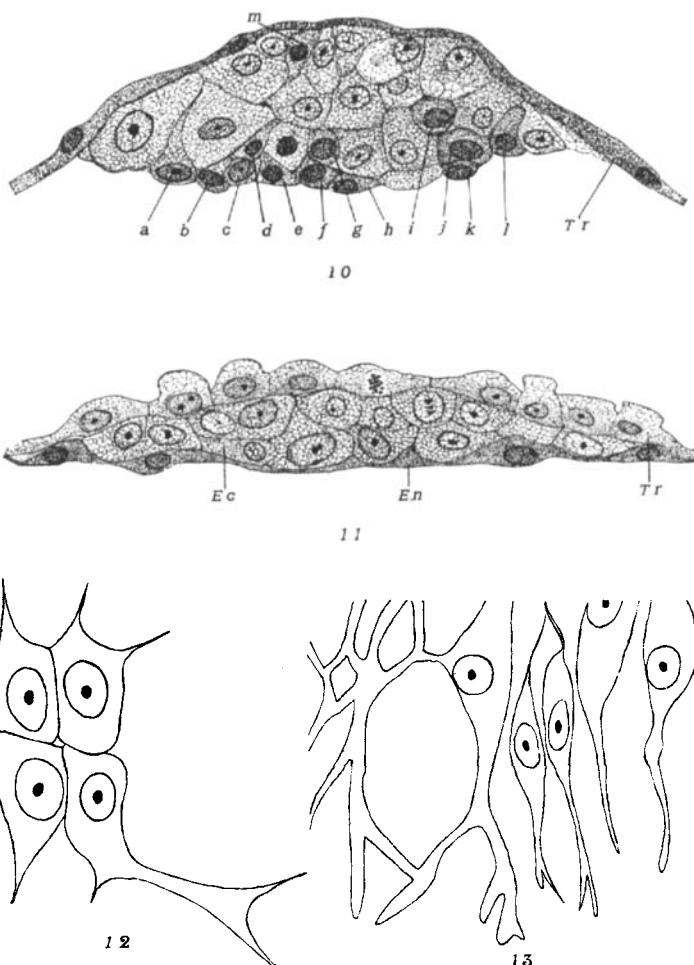


Fig. 10 Median section of the inner cell-mass of blastocyst No. 296. This specimen shows with special clearness the formation of the entoderm.  $\times 728$ .

Fig. 11 A section taken slightly to the side of the center of the inner cell-mass of blastocyst no. 311.  $\times 596$ .

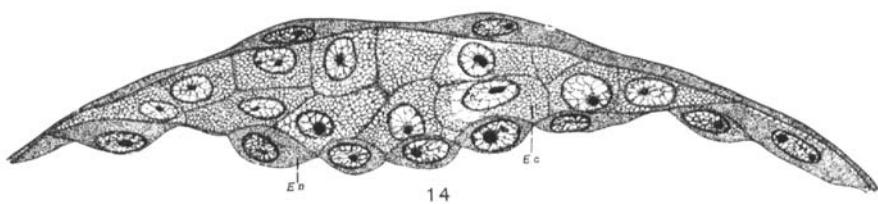
Fig. 12 Outline of the marginal entodermal cells from blastocyst No. 300.

Fig. 13 Marginal entodermal cells from blastocyst No. 320 (see plate 1).

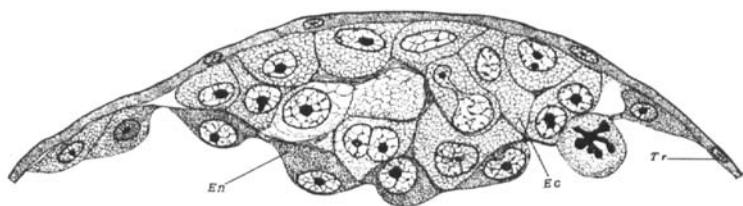
Fig. 14 Median section of the inner cell-mass of blastocyst No. 249.  $\times 1190$ .

Fig. 15 Median section of the embryonic spot of blastocyst No. 340.  $\times 1190$ .

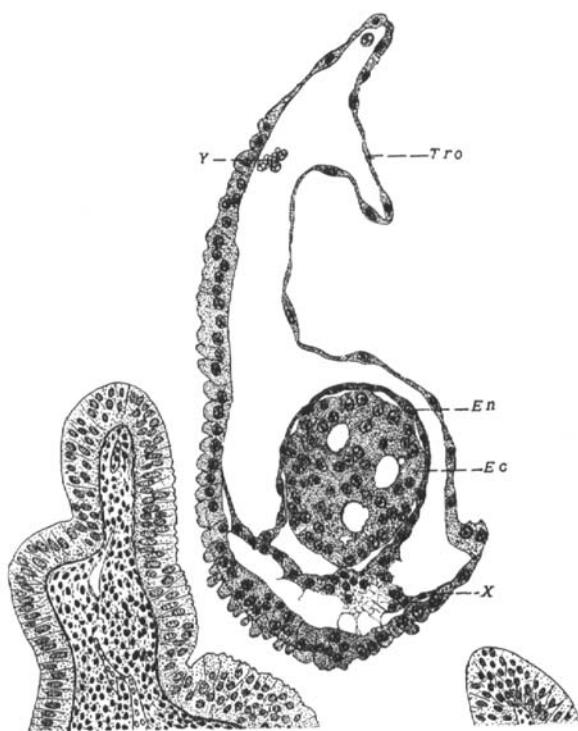
Fig. 16 A section passing through the center of the ectodermal mass of specimen No. 316. The sections are not quite perpendicular to the surface of the mucosa, and consequently the point of attachment does not show in this section (see plate 2).  $\times 218$ .



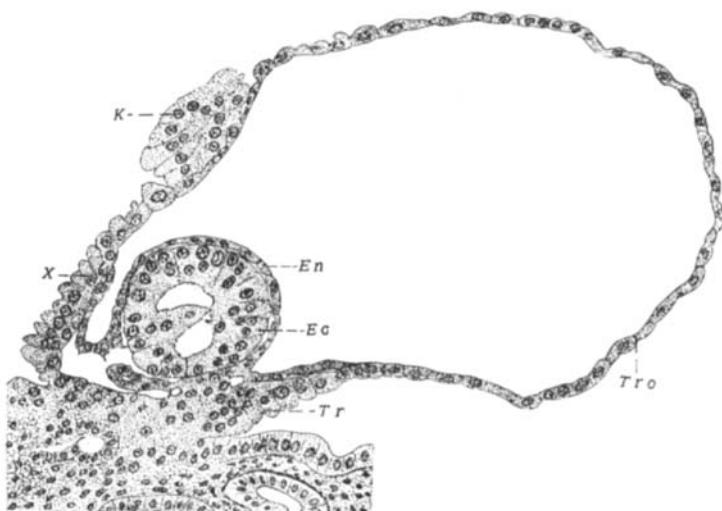
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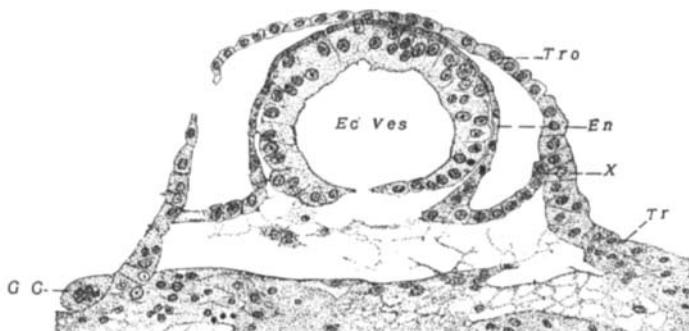
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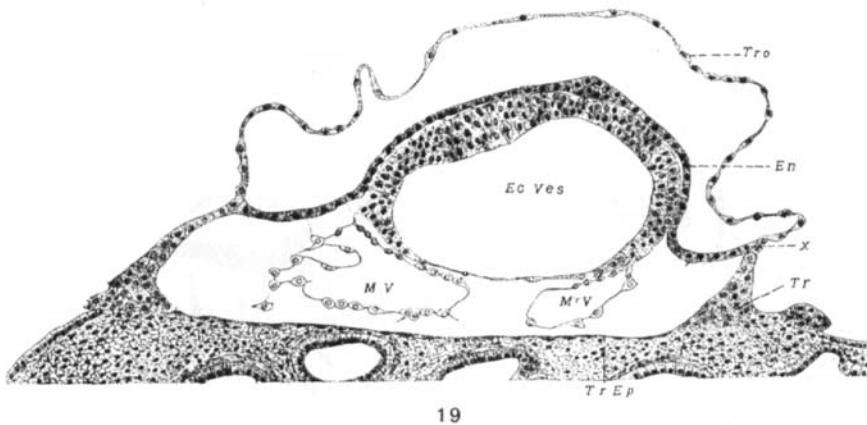
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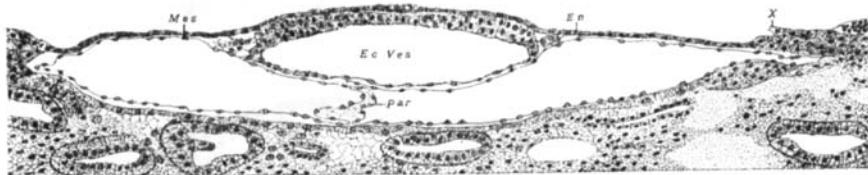
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Fig. 17 Median section of the ectodermal mass of specimen No. 332. This specimen is just a little older than the preceding, and shows with great clearness the manner in which the ectodermal mass is included within the entoderm. At *k* is a knot-like growth from the trophoblast. Note that the ectodermal mass is undergoing vacuolization to form a vesicle.  $\times 245$ .

Fig. 18 Median section of specimen No. 233. This shows the condition just at the close of the formation of the ectodermal vesicle (see text for description).  $\times 162$ .



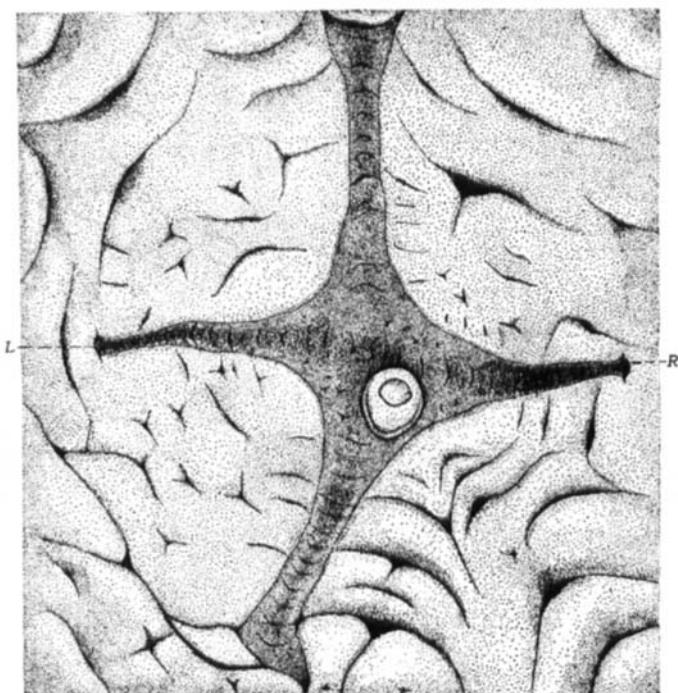
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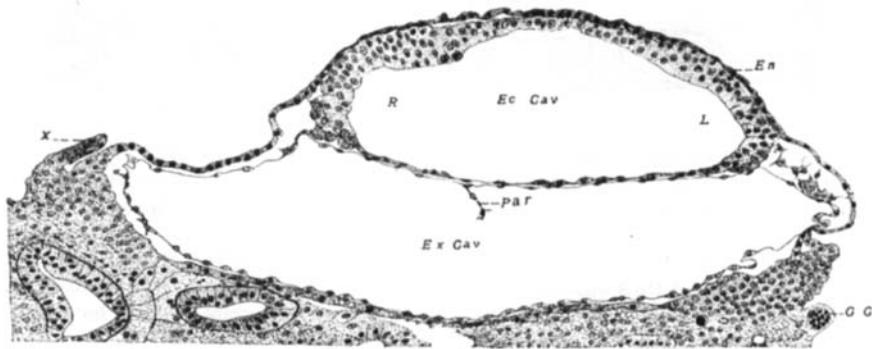
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Fig. 19 Median section in a right-left plane of specimen No. 234. The development of the mesoderm has made considerable progress, and the smaller of the two vesicles shows at 'x' the point where the entoderm joins the chorionic ectoderm (trophoblast).  $\times 142$ .

Fig. 20 A section similar to the preceding, from specimen No. 256. The vesicle has collapsed and thus appears very much flattened in the figure. The chorionic ectoderm has sloughed off and disappeared. The mesodermal vesicles have expanded until they now fill up the entire extraembryonic cavity.  $\times 113$ .



21



22

Fig. 21 Drawing of the inner surface of the fundus end of the uterus, from specimen No. 247. It shows the cross-shaped area, in the center of which is an attached vesicle. The vesicle has come from the right ovary and is attached on the right side of the placental area.  $\times 14.5$ .

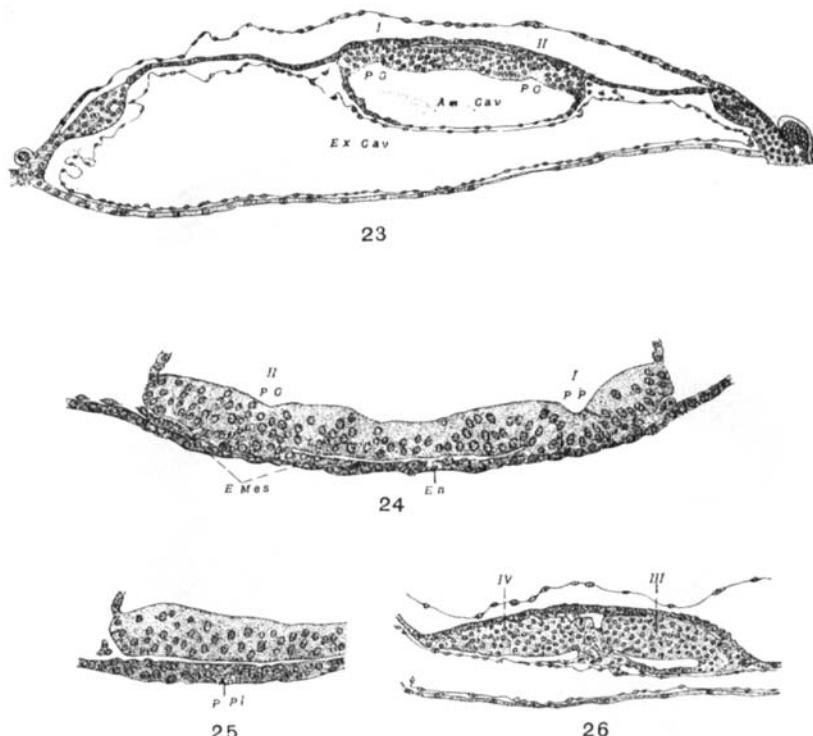


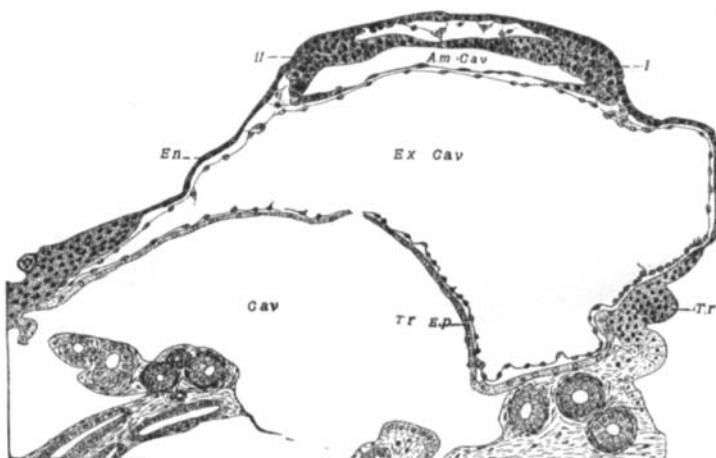
Fig. 22 A median section through plane *a* to *b*, figure 1. The right and left bays (*R* and *L*) of the primary buds are well shown in the figure. A remnant of the partition which previously separated the two mesodermal vesicles is seen at *par.*  $\times 130$ .

Fig. 23 The section through *e* to *f* of figure 2. It shows the transverse section across Embryos I and II. This specimen shows a rather rare condition for a blastocyst so far advanced in development, in that the chorionic ectoderm has not as yet disappeared. However, it shows many signs of degenerating.  $\times 71$ .

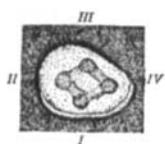
Fig. 24 Enlarged view of the embryonic portion of the ectodermal vesicle of the preceding figure. The figure is inverted in order to bring the dorsal sides of the embryonic primordia uppermost. The primitive streak region of each embryo is proliferating mesodermal cells (*E. Mes.*). In the case of Embryo I the section cuts across a pit-like depression (*P.P.*) of the primitive groove.  $\times 153$ .

Fig. 25 A portion of the section passing through the anterior end of Embryo II. It shows the ectoderm beginning to thicken preparatory to a proliferation of mesodermal cells. This region of the ectoderm corresponds to the protochordal plate of Hubrecht.  $\times 153$ .

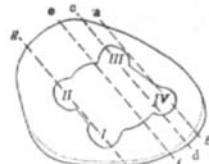
Fig. 26 A section across the tips of the secondary buds of Embryos III and IV.  $\times 153$ .



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Fig. 27 A section through *g* to *h* of figure 29. The buds of Embryos I and II are shown in section. The enlargement of the cavity (marked *Cav.*) lying below and to the left of the extraembryonic cavity is an artifact, caused by the lifting up of the Träger epithelium from the mucosa.  $\times 79$ .

Fig. 28 Sketch of the blastocyst No. 175, showing the four embryonic rudiments.  $\times 14.5$ .

Fig. 29 Diagram of the same specimen. The broken parallel lines show the planes of the sections illustrated in plate 10.  $\times 29$ .

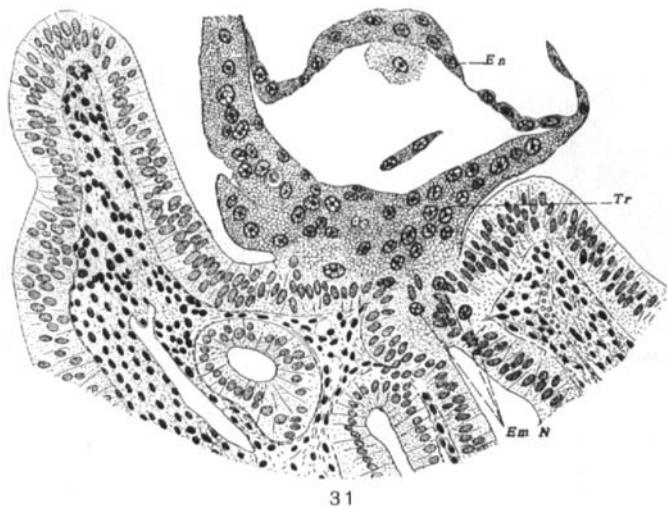
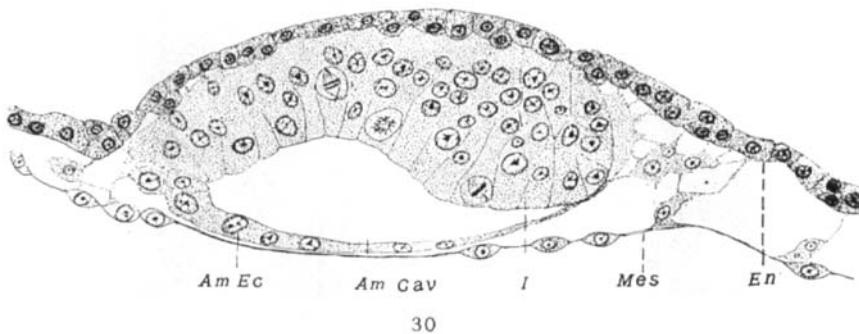
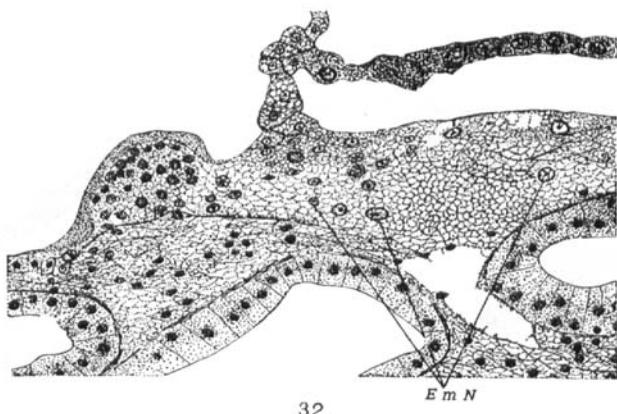
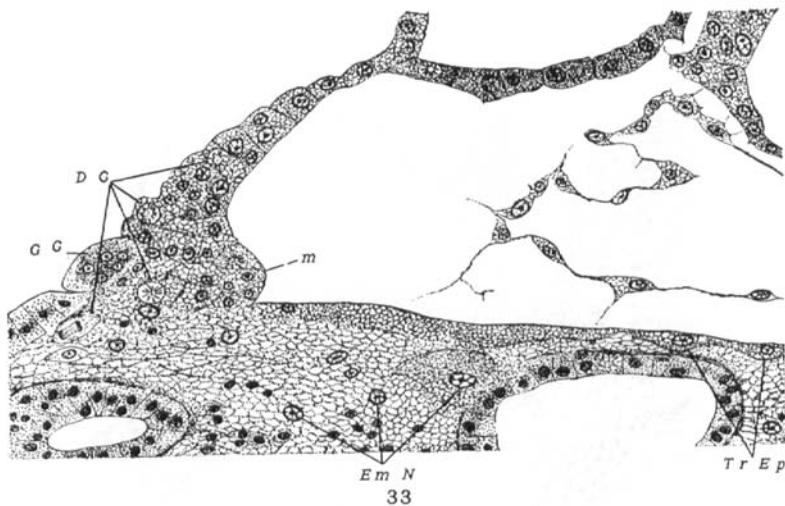


Fig. 30 Detailed drawing of a transverse section across bud I of specimen No. 257.  $\times 312$ .

Fig. 31 Section through the Träger region of blastocyst No. 316.  $\times 271$ .



32



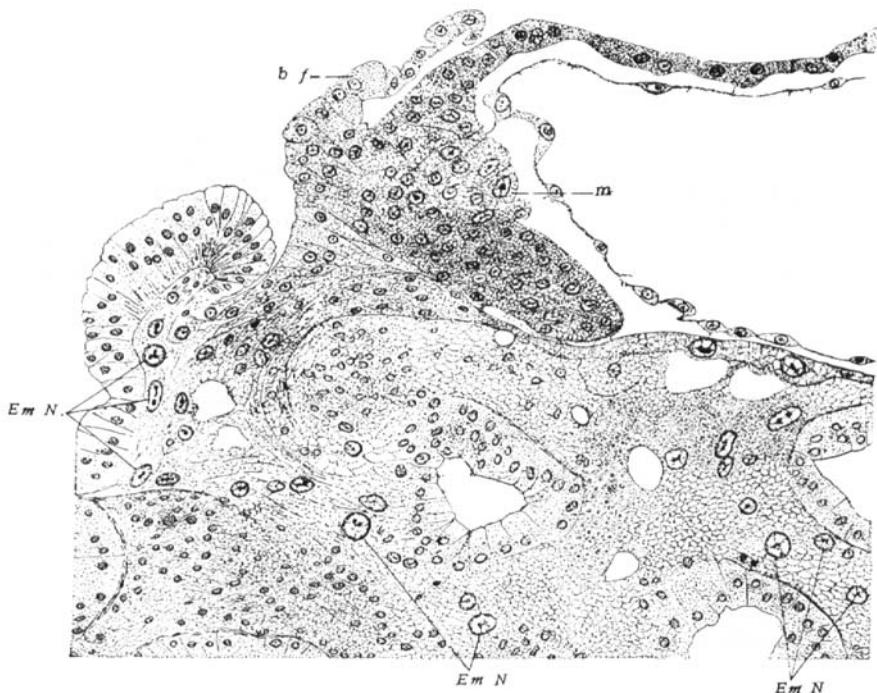
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Fig. 32 A portion of the left side of the section shown in figure 51.  $\times 306$ .

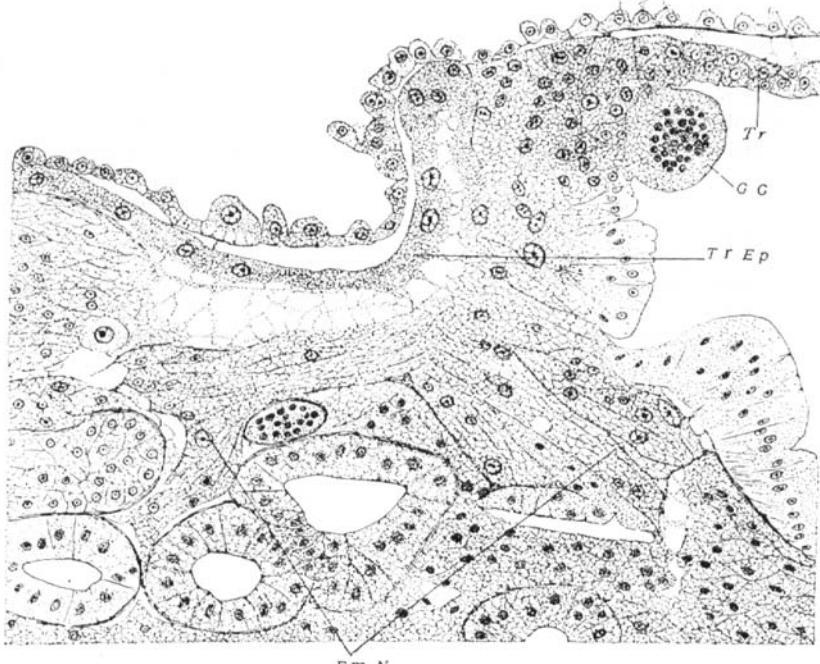
Fig. 33 A similar region from the section shown in figure 58. D.C., cells in mitosis; G.C., giant cell, which is frequently found at the base of the blastocyst. At *m* is the beginning of a mass of Träger cells which later extends up to receive the entoderm.  $\times 306$ .

Fig. 34 A portion of the left side of the section shown in figure 60. The mass at *m* now carries the entoderm, while the outer layer of Träger cells is seen as the basal fragment (*b.f.*). Note that the vacuoles are beginning to appear in the syncytium.  $\times 319$ .

Fig. 35 A portion of the right side of a section from specimen No. 175, plate 8. The Träger epithelium (*Tr.Ep.*) is quite well organized, and root-like growths of Träger tissue are seen extending into the mucosa at *Em*.  $\times 485$ .



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661

## DESCRIPTION OF PLATES

All of the photographic figures shown in these plates were made directly from the negative without retouching. The photographs were made by the use of the Bausch and Lomb combination micro-photographic and drawing apparatus. Various combinations of Zeiss objectives and oculars were used in the work. In practically every case the magnification is given after the description of the figure.

## ABBREVIATIONS

<i>C.A.V.</i> , common amniotic vesicle	<i>M.V.</i> , mesodermal vesicle
<i>Ec.</i> , ectoderm	<i>P.G.</i> , primitive groove
<i>En.</i> , entoderm	<i>P.P.</i> , primitive pit
<i>E.Mes.</i> , embryonic or primitive streak	<i>Tr.</i> , Träger
mesoderm	<i>Tr.Ep.</i> , Träger epithelium
<i>Em.N.</i> , embryonic nuclei	<i>Tro.</i> , trophoblast
<i>Ec.Ves.</i> , ectodermal vesicle	<i>I</i> , ventral embryo
<i>Ec.Cav.</i> , ectodermal cavity	<i>II</i> , right-lateral embryo
<i>G.C.</i> , giant cell	<i>III</i> , dorsal embryo
<i>Mes.</i> , mesothelium	<i>IV</i> , left-lateral embryo

## PLATE 1

### EXPLANATION OF FIGURES

36 Median section of blastocyst No. 310. This shows the knob-like inner cell-mass lying against the thin trophoblast.  $\times 186$ .

37 Median section of blastocyst No. 296. The deeply staining entodermal cells are seen in the act of migrating to the lower or inner side of the cell-mass.  $\times 186$ .

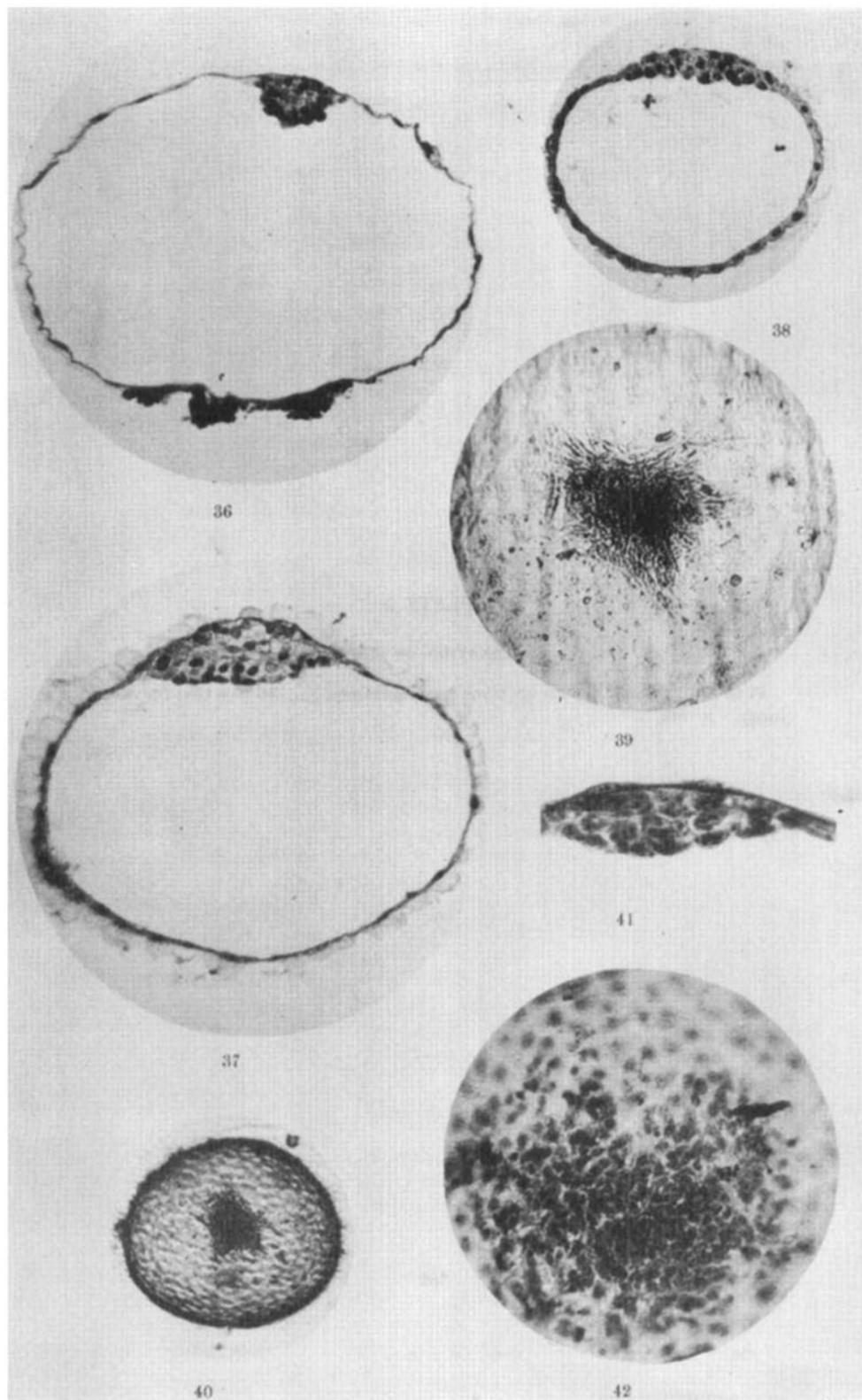
38 Median section of blastocyst No. 335. Note that the trophoblast has not yet become thinned out or attenuated.  $\times 186$ .

39 Enlarged view of the inner cell-mass of the blastocyst shown in the next figure. It clearly shows the pseudopod-like processes extending out from the marginal entodermal cells.  $\times 225$ .

40 Upper view of blastocyst No. 320. From an unstained glycerin jelly preparation.  $\times 90$ .

41 Median section of blastocyst No. 249. The section does not lie flat on the side, and since the microscope was focused on the trophoblastic cells, the inner cell-mass is slightly out of focus. Note how sharply Rauber's layer stands out from the mass.  $\times 417$ .

42 Enlarged view of the inner surface of the entoderm of blastocyst No. 300.



**PLATE 2**

**EXPLANATION OF FIGURES**

43 to 46 A series of four sections from specimen No. 316 (see text for descriptions).  $\times 148$ .

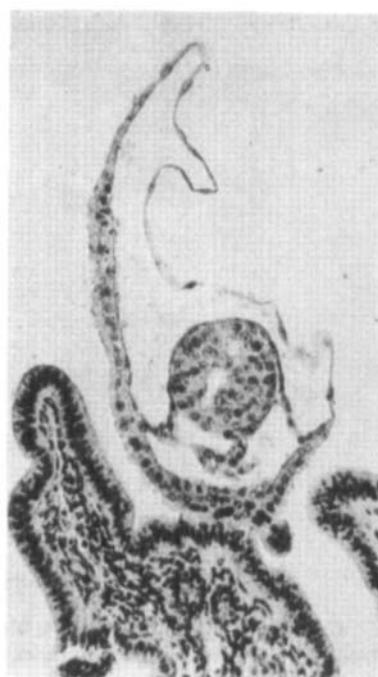
POLYEMBRYONIC DEVELOPMENT IN TATUSIA

J. T. PATTERSON

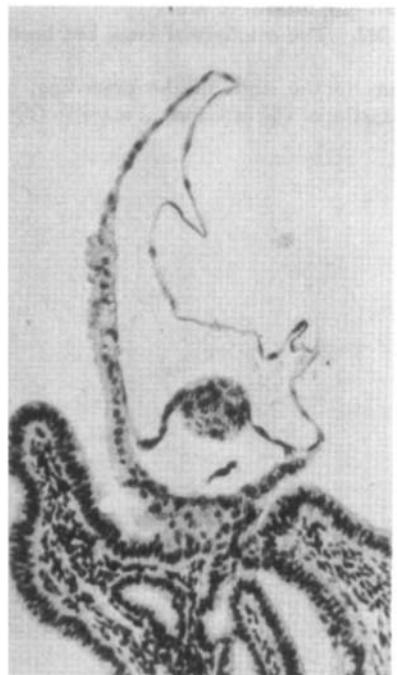
PLATE 2



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## PLATE 3

### EXPLANATION OF FIGURES

47 Portion of a section from blastocyst No. 311. It shows the well-organized layer of entoderm, which takes a deeper stain than does the overlying ectoderm.  $\times 417$ .

48 Similar section from specimen No. 340. This shows the first step in the rounding up of the ectoderm to form a ball-like mass.  $\times 416$ .

49 Median section of blastocyst No. 332. The ectodermal mass has become included within the entoderm.  $\times 149$ .

50 This section is taken four sections to the right of the preceding, and shows with especial clearness the vacuolization of the ectodermal mass to form a vesicle.  $\times 149$ .



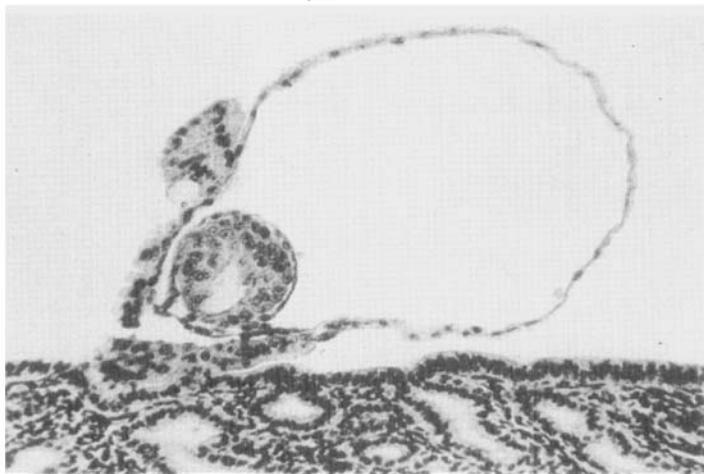
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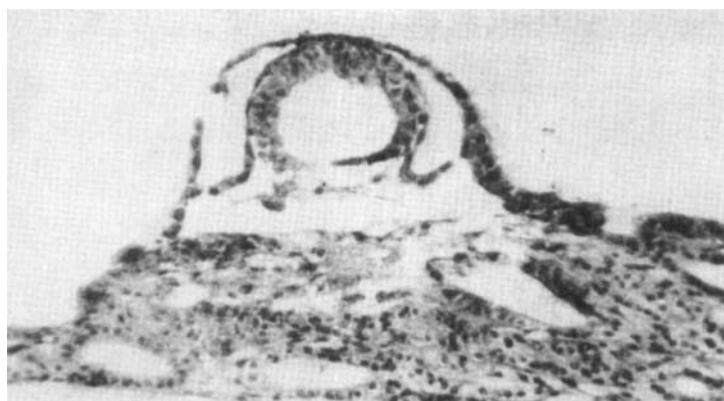


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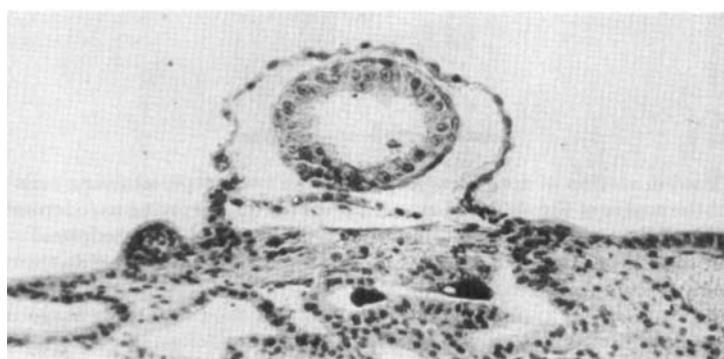
## PLATE 4

### EXPLANATION OF FIGURES

- 51 Median section of specimen No. 233. The ectodermal mass has become transformed into a vesicle, the lower wall of which has a small pore.  $\times 167$ .
- 52 This section lies ten sections to the right of the preceding.  $\times 167$ .
- 53 Section from blastocyst No. 329. This specimen was unfortunately crushed in transportation from the field to the laboratory, but the general relations of the different parts can be made out.  $\times 167$ .
- 54 Section from specimen No. 289. The relation of the different parts is particularly clear in this section. Note that the large extraembryonic cavity, which lies between the ectodermal vesicle and the mucosa, is entirely free from cells.  $\times 85$ .



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## PLATE 5

### EXPLANATION OF FIGURES

55 Median section of specimen No. 289. The two deeply-staining cells lying at about the center of the extraembryonic cavity are degenerating entodermal cells the single cell situated toward the right side of this cavity is of ectodermal origin, and indicates the beginning of a cell proliferation which will give rise to the extraembryonic mesoderm.  $\times 103$ .

56 Median section from specimen No. 298. It shows an early stage in the development of the mesoderin, which appears in the form of small vesicles.  $\times 85$ .

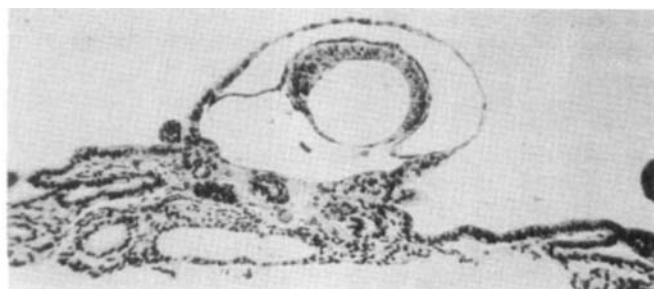
57 A section lying four sections to the left of the preceding, and showing well-formed but small mesoderm vesicles.  $\times 106$ .

58 Median section, taken in a right-left plane, of No. 234. This shows two relatively large mesodermal vesicles, which have been formed by a fusion of smaller vesicles, such as appear in the preceding figure.  $\times 106$ .

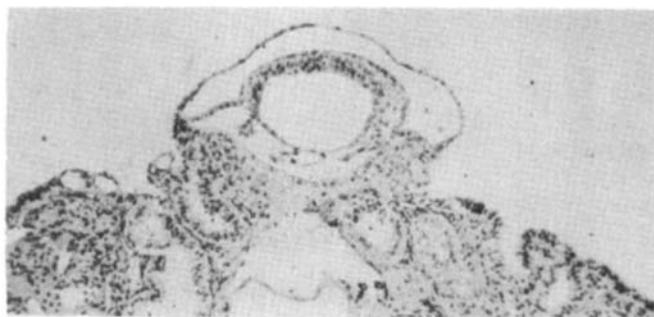
POLYEMBRYONIC DEVELOPMENT IN TATUSIA

J. T. PATTERSON

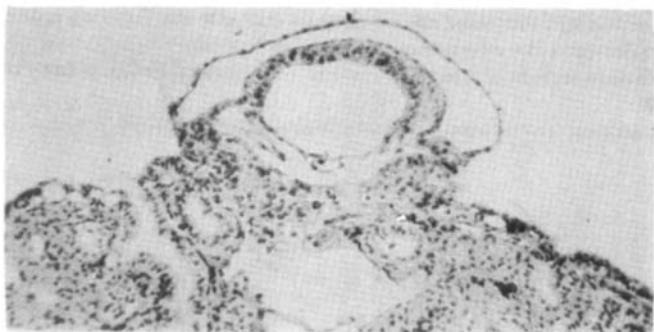
PLATE 5



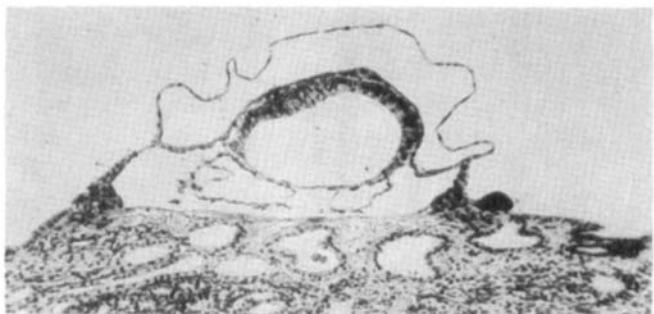
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## PLATE 6

### EXPLANATION OF FIGURES

59 Median section, taken in a right-left plane, of specimen No. 256. The two mesodermal vesicles have expanded until they now fill up the entire large extra-embryonic cavity; but their adjacent walls still remain, forming a double-walled partition between the cavities of the vesicles.  $\times 95$ .

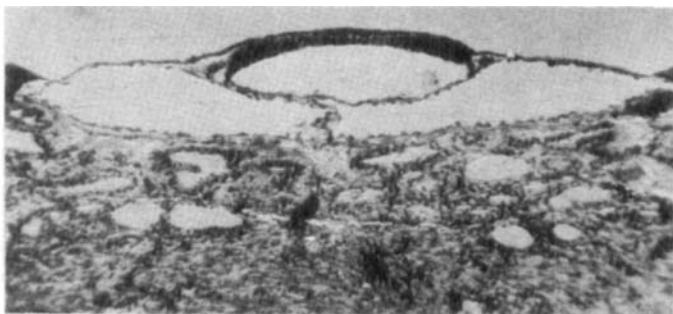
60 Median section of specimen No. 247. It passes through plane *a* to *b* of figure 1.  $\times 90$ .

61 A section taken through plane *c* to *d* of figure 1.  $\times 90$ .

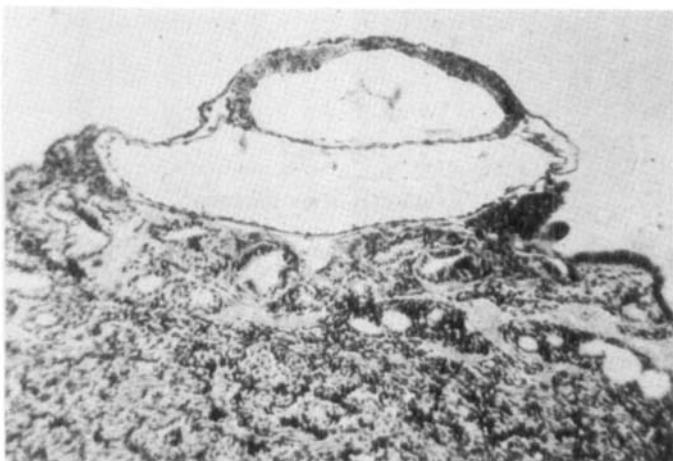
POLYEMBRYONIC DEVELOPMENT IN TATUSIA

J. T. PATTERSON

PLATE 6



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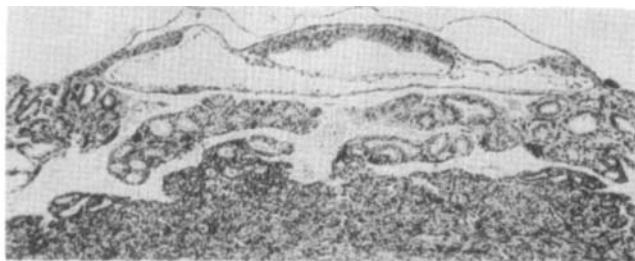


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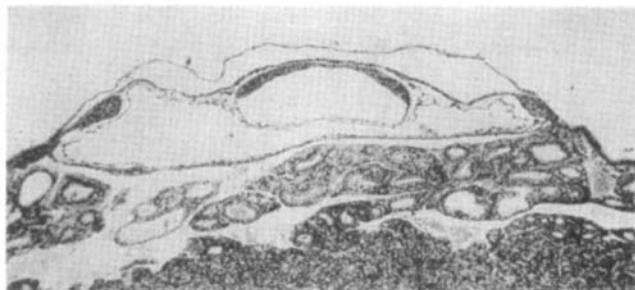
## PLATE 7

### EXPLANATION OF FIGURES

- 62 to 65 A series of four sections from specimen No. 290; all  $\times 46$ .  
62 Taken through plane *a* to *b*, figure 2.  
63 Taken through plane *c* to *d*, figure 2.  
64 Taken through plane *e* to *f*, figure 2.  
65 Taken through plane *g* to *h*, figure 2.



62



63



64

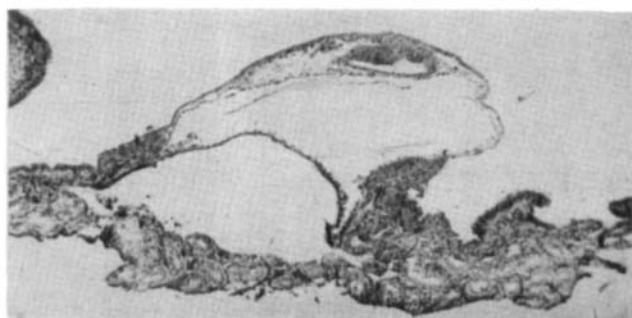


65

## PLATE 8

### EXPLANATION OF FIGURES

- 66 to 69 A series of four sections from specimen No. 175; all  $\times 56$ .  
66 Taken through plane *a* to *b*, figure 29.  
67 Taken through plane *c* to *d*, figure 29.  
68 Taken through plane *e* to *f*, figure 29.  
69 Taken through plane *g* to *h*, figure 29.



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## PLATE 9

### EXPLANATION OF FIGURES

- 70 to 74 A series of five sections from specimen No. 257; all  $\times$  63.  
70 Taken through plane *a* to *b*, figure 3.  
71 Taken through plane *c* to *d*, figure 3.  
72 Taken through plane *e* to *f*, figure 3.  
73 Taken through plane *g* to *h*, figure 3.  
74 Taken through plane *i* to *j*, figure 3.

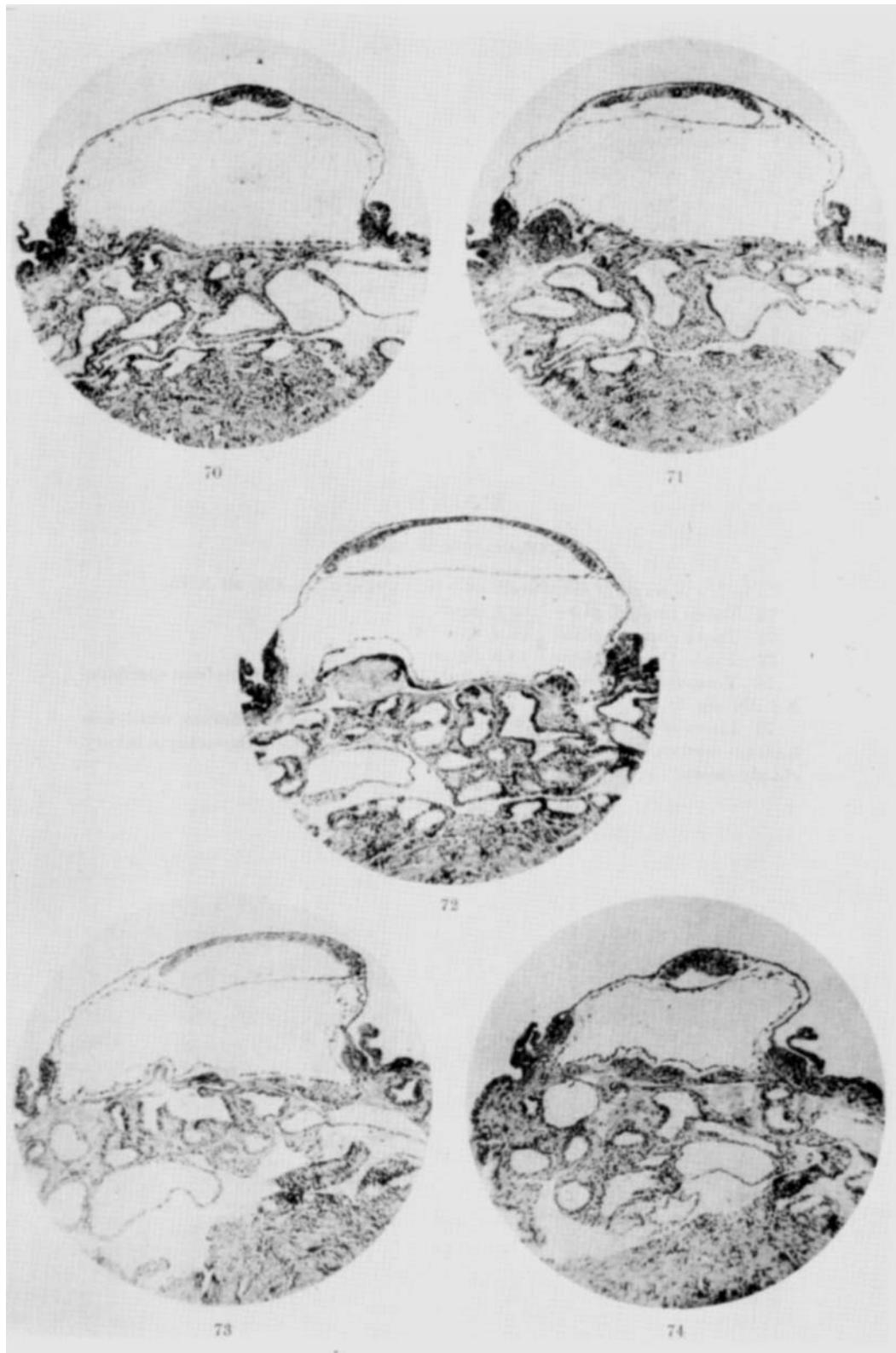
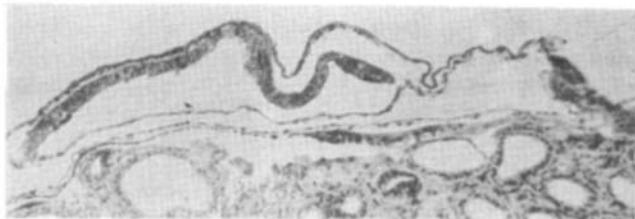


PLATE 10

EXPLANATION OF FIGURES

- 75 to 77 A series of three sections from specimen No. 170; all  $\times 75$ .  
75 Taken through plane *c* to *f*, figure 4.  
76 Taken through plane *c* to *d*, figure 4.  
77 Taken through plane *a* to *b*, figure 4.  
78 Transverse section through the middle of one of the embryos from specimen No. 276 (fig. 5).  
79 Litter of four embryos attached to the placentae of the chorion, which has been cut open on the ventral side. The paired arrangement of the embryos is very clearly shown.  $\times \frac{1}{3}$ .



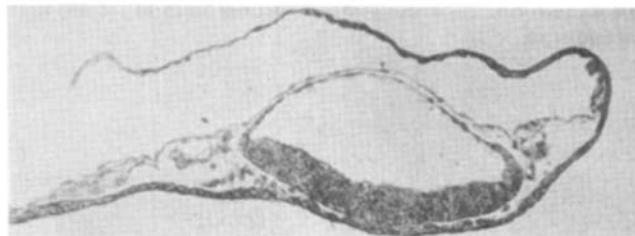
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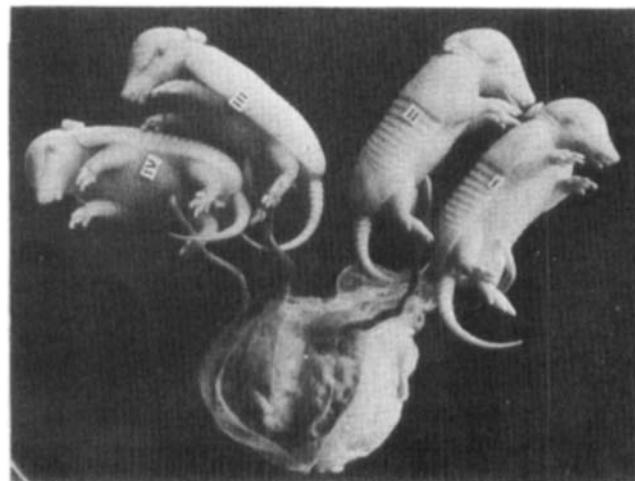
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78



## PLATE 11

### EXPLANATION OF FIGURES

80 to 84 A series of five sections from specimen No. 226. The sections are cut almost transversely to the long axes of the embryos, that is, parallel to the surface of the mucosa (see text for descriptions). Figures 80 to 81,  $\times 46$ ; figures 82 to 83,  $\times 42$ ; and figure 84,  $\times 37$ .

