SOME PHASES OF THE GASTRULATION OF THE HORNED TOAD, PHRYNOSOMA CORNUTUM HARLAN.

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WITH 15 TEXT FIGURES.

The following account of the gastrulation of this very interesting iguanid is the outcome of investigations carried on at the University of Cincinnati in 1899 and 1900 and at Trinity College in 1900 and 1901. The embryos were collected at Austin, Texas, in 1892-1894. Except for the cleavage and the beginning of gastrulation the collection embraces the complete embryology of *Phrynosoma cornutum*, but in this paper we present only the general phenomena of the gastrulation as interpreted from the stages in our possession. The breeding and nest building habits of the horned toad have been described by Edwards, 96. Breeding takes place in the months of June, July and August. Contrary to all previous accounts in which species of the genus *Phrynosoma* are given as viviparous¹ it was observed that at least in the case of *P. cornutum* at Austin, Texas, the eggs, numbering in some instances as many as twenty-five, are deposited in nests formed in a chamber at the end of a tunnel in the ground which the female burrows, and then, after laying the eggs, carefully refills with the loose pellets of earth. At the time the eggs are laid the embryo has attained the stage in ontogeny equivalent to that represented by Peter, 04, Taf. II, Fig. 17, as N. T. Nr. 68 for *Lacerta agilis*. The *Phrynosoma* embryo when it leaves the oviduct is 2 mm. in its greatest length. The head bend forms a wide acute angle at the projecting mid-brain, while the neck bend forms an obtuse angle. The body is slightly curved, the caudal end being twisted around to the right. In the open space of half the length of the body between the tip of the fore-brain and the tail lie the well marked, distended allantois and the strongly curved, prominent heart.

In order to obtain the earlier stages, it is necessary to take the eggs

¹ This error, repeated by Gadow, 01, p. 533, was corrected by Edwards, 03, p. 826.

from the oviduct of a gravid female immediately after she has been chloroformed. The oblong eggs are cream-colored and when laid the soft, moist, semi-transparent membranous shell allows the embryonic area to show through, forming a dark pinkish oval area on the upper side of the egg. The egg shell becomes tough on drying, but not brittle or stiff. To fix the embryos free from yolk and separated from the egg shell, a disk somewhat larger than the embryonic area was cut out. In removing this a considerable portion of the yolk immediately beneath was carried with it to sustain the embryo until it could be supported on all sides by physiological salt solution. By the careful use of a current from a pipette, the yolk was removed and then the shell membrane and the vitelline membrane. Sometimes, in very early stages, it was found desirable to allow the shell membrane to remain on the blastoderm for its support. Usually a drawing was made of the unstained embryo with the aid of about sixty diameters magnification, in order to facilitate the interpretation of sections. In general, Fleming's mixture of chromic-aceto-osmic acid was employed for fixing followed by successive alcohols. A modification of Mayer's hæmalum was found superior to hæmatoxylin and other hæmatin stains, both for sections and specimens *in toto*. For the latter Mayer's hæmalum diluted with twenty parts of ammonia alum was employed. The specimen was decolorized in 1-10 of 1% hydrochloric acid made up in 70% alcohol. Alcoholic cochineal also gave good results *in toto*. Benzopurpurin was advantageous in older stages. Orange G was used as a plasma stain in sections. Clearing was accomplished by the use of anilin, or clove oil, removed subsequently by xylol.

Owing to the radially symmetrical appearance of the embryonic area in the earliest stages, they were extremely difficult to orient. They were embedded in celloidin and then the celloidin was pared down until the embryos could be observed under the low power of a microscope when triangular blocks were cut with definite relation to the anterior and posterior end of the blastoderm. These were reëMBEDDED when sectioned. The celloidin also protected the delicate embryos which had become brittle after several years in alcohol.

The youngest embryo in this collection has passed through cleavage and the first phase of gastrulation being in the second phase of gastrulation as first worked out for the lizard by Wenckebach, 91, p. 75.

This stage is represented in surface view in Fig. 1. Superficially it resembles a like stage of *Lacerta viridis*, according to the drawings of Will, 95, b, Fig. 6, Pl. 1; also of the turtle embryo as represented by Mitsukuri, 94, Fig. 1, Pl. 6, and of Tuatara (*Hatteria*) as figured by Dendy, 99, Fig. 1. The embryonic area is pushed up above the surrounding

blastoderm in the form of a cap with vertical margins leveled toward the center of the crown and having a deep recess in the posterior edge where the blastopore is located. The length of the area is about 4 mm. The width is slightly less. At the anterior margin the cap tapers to a round point which indicates more rapid growth in this plane. The recess at the posterior edge indicates either an ingrowth of the upper layer cells at this point or a backward movement of parts on either side of it. Sections prove that the former is true.

This stage is represented as seen in longitudinal section in Figs. 2 and 3. These sections are slightly diagonal. Fig. 2 passes through the blasto-

FIG. 1. Dorsal view of a very early stage in which probably the mesoblast sac has not broken through into the subgerminal space. *a.*, anterior; *aa. em.*, area embryonic; *aa. pel.*, area pellucida; *bl. 'po.*, blastopore; *p.*, posterior; $\times 74$ diameters.

pore, but runs to the left of the median plane in the anterior region of the embryonic area. The extent of this rotation may be judged from the fact that Figs. 2 and 3 are the 2d and 3d sections which pass through the embryonic area, there being 9 sections included in this area. All the structures of interest are to be found in these two sections.

The elevation of the cap above the surrounding blastoderm is apparently due to the rapid increase in thickness and extent of the epiblast over this area. The accumulation of the mesoblast may take part in this elevation, but the absence of mesoblast in the greater part of the anterior

FIG. 3.

FIGS. 2 AND 3. Longitudinal sections of the blastoderm seen in surface view in Fig. 1. Fig. 2 is median through the blastopore but runs to the left of the middle line in front. Fig. 3 is the next section to the right of Fig. 2. In Fig. 2, *a*, *b*, and *la*, *pr.*, and in Fig. 3, *a*, *b*, *c*, are represented tissues which appear only at deep focus. *bl.*, *po.*, blastopore; *e.*, *tbl.*, epiblast; *h.*, *tbl.*, hypoblast; *la.*, *n.*, neural plate; *la.*, *pr.*, primitive plate; *ms.*, *tbl.*, mesoblast; *prc.*, *ce.*, head process; $\times 200$ diameters.

embryonic area proves that some other agent maintains the elevated position of the thickened epiblast.

As in other reptiles the upper and lower layer are connected by a mass of mesoblast at the posterior edge of the embryonic area. Here by the invagination of the primitive plate of Will is formed the mesoblast sac (Mesodermsäckchen of O. Hertwig, 03, p. 828). That all of the mesoblast in Figs. 2 and 3 was derived from the proliferation and invagination of the region of the primitive plate and blastopore, can not be positively affirmed, yet it seems highly probable. The mesoblast at the anterior margin in Fig. 3 is in direct connection with the mesoblast under the primitive plate.

The mesoblast cell masses are continuous with the epiblast of the posterior and lateral lips of the blastopore which leaves little doubt as to their origin. From these two sources there is a lateral and a forward extension of the mesoblast cell masses. From the floor of the mesoblast sac one mass extends forward in the middle region (Fig. 2, ms'bl.^{vi}) It is continuous laterally with the lateral extensions, the floor of the mesoblast sac contributing to the lower portion of these also. The inverted epiblast, or lateral roof of the mesoblast sac is continued laterally and forward by a mass of mesoblast (Fig. 3, ms'bl.ⁱⁱⁱ) having a high position. Laterally this mesoblast bends down and back (Fig. 2, ms'bl.ⁱⁱⁱ) to unite with the mesoblast from the floor of the mesoblast sac (Fig. 2, ms'bl.ⁱⁱⁱ). It thus forms a pouch on either side which is continuous posteriorly with the mesoblast sac in the median line. The floor of the mesoblast sac is continued anteriorly for a short distance under the chorda anlage into a sheet of mesoblast. In a middle position it spreads laterally over the lateral pouch above mentioned, while farther back, as the floor of the mesoblast sac, it is continuous with the ventral wall of the lateral pouches.

Under the region designated primitive plate (Fig. 2, la. pr.) the mesoblast is in the form of a network, and, while it seems to be spreading from these centers of origin, it is not being encroached upon by new additions, or proliferation, *inter se*. Mesoblast of this nature can be traced from the hollow pouches extending laterally on either side of the primitive streak, to the anterior margin of the embryonic area where it fills in the space between the epiblast and hypoblast (Fig. 3 ms'bl.ⁱⁱⁱ). According to Will a similar forward growth of mesoblast on each side of the chorda takes place in *Lacerta agilis*, *L. muralis* and a like condition exists in the turtle (Mitsukuri, 94), snake (Will, 98) and in *Sphenodon* (Dendy, 99).

The mesoblast sac becomes a canal reaching far into the mass of meso-

blast, almost to the middle of the embryonic area. At the mouth it is funnel-shaped, being wider perhaps laterally than vertically. Its dorsal wall is the thick infolded epiblast destined to become the chorda. The epiblast of the depressed ventral wall of the blastopore becomes mesoblast deeper in the canal, for this mesoblast is produced by the forward growth of epiblast cells. Since the dorsal and ventral walls of the mesoblast canal contribute to the lateral mesoblast by infolding and lateral growth, the mesoblast sac may be regarded as having no lateral limitations, but extending between the above named mesoblast anlagen. However, the sections of this stage only indicate this for a short distance in front of the blastopore. The head process is four or five cells thick where it is continuous with the epiblast at the blastopore. It is continuous laterally at this point with the ectodermic folds that give rise on either side to the dorsal limbs of the mesoblastic pouches. Anteriorly it becomes a wide belt of cells, three or four deep, and thin at the edges (Fig. 3, *pr. ce.*) extending quite to the anterior limit of the neural plate. As in Fig. 3, anterior sections do not have mesoderm adjacent to the chorda laterally. A part of the tissue under the chorda in Fig. 3 is mesoblast (Fig. 3, *ms'bl.^{vi}*) of primary origin, but under the greater part of it is mesoblast (Fig. 3, *ms'bl.ⁱⁱ*) which is derived from the primary mesoblast. In the adjacent sections the hypoblast is easily traceable to the limits of the embryonic area and there is no mesoblast lateral to the chorda in the forward end.

In Figs. 2 and 3 the epiblast represented is not all in the same plane (*e'bl.ⁱⁱ* and *e'bl.ⁱⁱⁱ*); *e'bl.ⁱⁱⁱ* is cut in a diagonal plane and *e'bl.ⁱⁱ* is cut vertically. In Fig. 2 (*a, b* and *la. pr.*) and Fig. 3 (*a, b, c*) are represented tissues which appear only at deep focus. The epiblast over the whole top of the embryonic cap is four or five cells thick, but it thins out rapidly toward the base of the uprising wall, becoming a thin one-celled layer in the extra-embryonic area (Figs. 2 and 3, *e'bl.ⁱ*). The hypoblast is uniformly one cell thick over the whole embryonic area, except under the chorda where it appears to be two or more cells thick, but this condition is of limited extent. Extra-embryonic mesoblast separates these two germ layers but a short distance from the embryonic area (Fig. 2 *ms'bl.ⁱ* Fig. 3, *ms'bl.ⁱⁱⁱⁱ*). From these observations we may conclude that at a time when the epiblast is in the form of a much thickened cap the mesoblast sac forms a canal stretching forward into the mass of tissues that are then accumulating at the posterior part of the embryonic cap and perhaps opening into the sub-germinal cavity in front of this mass of tissues. The mesoblast which is being produced around the blastopore, stretches antero-laterally from it in the forms of two hollow sacs of

closely packed cells, one on each side, which act as feeders to surrounding parts, there being an extension of it along each lateral margin to a position well in front of the embryonic cap. The cavities of these sacs are in communication with the blastopore. The epiblast turns in at the blastopore and gives rise to the chorda and mesoblast. The latter also receives accessions below from the primitive plate region, in fact from epiblast cells of the floor of the mesoblast sac as we shall endeavor to prove later.

FIG. 4. Longitudinal section of an embryo in which the cap-like condition is less marked but in which the mesoblast sac has not yet fused with, or broken through, the hypoblast. *bl'.po.*, blastopore; *cd.d.*, chorda dorsalis; *e'.bl.*, epiblast; *h'.bl.*, hypoblast; *la pr.*, primitive plate; *ms'.bl.*, mesoblast; $\times 166\frac{2}{3}$ diameters.

The next oldest blastoderm in the collection was sectioned lengthwise. In the stage just described we have not been able to affirm positively that the mesoblast sac does not communicate with the subgerminal space, but in the second stage (Fig. 4) the hypoblast is unbroken. It differs from the younger stage in the absence of the cap-like elevation, in the more compact and mature condition of the primitive plate, and in the absence of the mesoblastic pouches so characteristic of the early stage. They are already flattened and their cavity all but obliterated. The chorda is relatively shorter than in the embryo represented in Figs. 2 and 3, but it is actually of about the same length while the whole blastoderm is about

two and one-half to three times as long as the blastoderm of Figs. 2 and 3. It is very nearly identical to Will's Fig. 36, Taf. 6, which is a section of the embryo represented by him in Fig. 6, Taf. 1, above referred to as equivalent to *Phrynosoma*, Fig. 1. The second stage has three separate solid masses of mesoblast proliferation and ingrowth. Two are from either side of the primitive plate and may be traced forward on either side. The cells become scattered at the edges of the growth. Some of these scattered cells may be seen between the thin epiblast and hypoblast at the anterior margin of the embryonic area, but not in the middle line.

FIG. 5. Dorsal view of a blastoderm older than Fig. 1. The elevation of the embryonic area has given place to a flat plate and the blastopore has changed shape. *aa.em.*, area embryonic; *bl.'po.*, blastopore; *glb.vt.*, yolk spheres; *la.pr.*, primitive plate; *marg.bl.'po.*, margin of blastopore; *mur.g.*, germinal wall; $\times 74$ diameters.

Here the epiblast and hypoblast are closely adherent both in and outside of the embryonic area. The third mass of mesoblast growth is from the ventral wall of the notochord. The primitive plate sends forward a mass of cells (Fig. 4, *ms'bl.vi*) detached from the hypoblast and extending almost as far as does the chorda. It is comparable to *ms'bl.vi* of Fig. 2. We do not regard it as a permanent source of anterior mesoblast, rather as evidence of a mesoblast sac. After very careful search it has

been impossible to discover unmistakable mesoblast cells outside the immediate vicinity of that from the embryonic area. On all sides are crowded hypoblast cells derived from the germinal wall, to which the thin epiblast is closely applied. In several instances mesoblast cells have been seen some distance from the embryonic mesoblast yet so similar in position to isolated mesoblast cells that are undoubtedly migrants from the embryonic area, that it seems safe to assume that they, too, have wandered. All cases of this character have been in the vicinity of isolated masses of wandering mesoblast. There is no evidence that up to the age of these two embryos, mesoblast arises elsewhere than around the blastopore. In subsequent stages all mesoblast found in the extra embryonic area must be suspected of having its origin in the detached cells which we have already called attention to some distance from the cell mass to which they originally belonged.

The caplike elevation of the blastoderm of *Phrynosoma* gives place to a flat embryonic area. Fig. 5 is a dorsal view of this stage. In this blastoderm the embryonic area was .95 mm. long and .89 mm. wide. A few large yolk spherules can be seen through the blastoderm in front of the blastopore, whence they extend forward over an ever widening area and may be seen around the rim of the embryonic area, except on the posterior side. They may possibly owe their size and form to the action of reagents, but their peculiar distribution is, without doubt, due to some structural condition before fixation. It is a very common thing to find in sections, large yolk laden cells engrossed by the hypoblast adjacent to the germinal wall, and beneath this hypoblast, large yolk masses. Hence it seems natural to regard the yolk masses in Fig. 5 as evidence of the rapid addition of yolk-laden formative cells to the hypoblast from the adjacent yolk mass. From the surface view there is evidence of greater thickness of the blastoderm back of the blastopore than is the case in the youngest stage. This is due rather to the spread of mesoblast than to thickening of the epiblast. The blastopore is less V-shaped than earlier and more like a half-moon. The depression between the primitive plate cells within the blastopore and the epiblast wall which rises in front of it represents the posterior end of the mesoblast sac. It is V-shaped in section (Fig. 10) opening postero-medially. The inturned edges of the epiblast along the entire length of the blastopore imply a process of ectodermic invagination along the whole margin. The surface view scarcely suggests the part the primitive plate is taking in this process.

This same embryo was subsequently sectioned in a longitudinal plane.

Fig. 6 is a semi-diagrammatic drawing of a sagittal section, and Fig. 7 of the next section to one side. In the latter the continuity of the epiblast and primitive plate mesoblast with the anterior mesoblast confirms what has been seen in the surface view with regard to the infolding epiblast and establishes the lateral growth of the primitive plate mesoblast as well. Hence, both the posterior and anterior lips of the blastopore are undergoing a process of invagination. The yolk masses can be seen adhering to the hypoblast and anteriorly it is receiving additions of large yolk-laden cells from the germinal wall. At this stage the mesoblast

FIG. 6.

FIG. 7.

FIGS. 6 AND 7. Longitudinal sections of the embryo represented in Fig. 5. Fig. 6 is sagittal and Fig. 7 is the third section to the right of Fig. 6. *can.n'ent.*, canalis neurentericus; *e'.bl.*, epiblast; *glb.vt.*, yolk spheres; *h'.bl.*, hypoblast; *la.pr.*, primitive plate; *ms'.bl.*, mesoblast; *prc.ce.*, head process; $\times 109$ diameters.

canal is an open passage into the subgerminal cavity, the canalis neurentericus, (cf. Hertwig, 03, pp. 832-4). The head process has become the lower layer along the middle line. This is due, no doubt, to the fact that its elongation has kept pace with the increase in length of the epiblast, hence after fusing with the hypoblast in a position near the middle of the embryonic area at the time, this point is carried forward relatively by the rapid elongation of the head process. The hypoblast has not yet grown together under the head process as it does later. While our ob-

FIG. 8.

FIG. 9.

FIGS. 8 AND 9. Longitudinal sections of a stage still older than Fig. 5. Fig. 8 is median. The embryonic area is still slightly elevated. Fig. 9 is the next section to the right. It shows the epiblast turning in to form mesoblast at the very edge of where it becomes chorda. *a.*, anterior; *bl. po.*, blastopore; *cd. d.*, chorda dorsalis; *e. bl.*, epiblast; *h. bl.*, hypoblast; *la. pr.*, primitive plate; *mur. g.*, germinal wall; *p.*, posterior; *t. t.*, yolk. Fig. 8, $\times 200$ diameters; Fig. 9, $\times 200$ diameters.

servations do not confirm the hypothesis, it seems probable that along with the process just described, the space from the median hypoblast in front of the primitive plate to the point where the chorda is in contact with the hypoblast anteriorly is due at first to the degeneration of the hypoblast and lower layer of the chorda invagination which Will, 93, found in the Gecko and represents in Figs. 57 and 58, Pl. 9. Then the rapid forward growth of the chorda carries its anterior contact still farther from the primitive plate hypoblast. In Fig. 7 one may see the anterior reach of the lateral mesoblast which has established the five or six mesoblast cells that are to be seen in the middle line in front of the embryonic area in Fig. 6.

Longitudinal sections of a stage which is a little younger than this are represented in Figs. 8 and 9. The cap-like form still persists. Other conditions are essentially the same as in the embryo just described. The head process is fused with the hypoblast and extends to the germinal wall, but the histological differentiation has not taken place in the anterior third of its length. In Fig. 9 the continuity of the epiblast and primitive streak mesoblast is not different from conditions described in the older embryo of this stage. The extension of the primitive plate cells under the chorda verifies the assertion previously made that the primitive plate mesoblast proliferates or grows laterally and forward as the ventral wall of the mesoblast sac. By so doing it is the counterpart of the epiblast on the dorsal wall in a single invagination. In this stage the network of mesoblast cells to be seen under the primitive plate in Fig. 2, has been replaced by the solid tissue. The condition persists, however, posterior to this region. Three or four sections from the median line this embryo has the same appearance as in the older specimen represented in Fig. 7. Cross sections of this stage are reproduced in Figs. 10-15

Fig. 10 passes through the blastopore where the V-shaped depressions mentioned in connection with Fig. 5 are prominent. Here the epiblast may be seen turning into these depressions and fusing in this section with the epiblast of the floor of the depression in the formation of a lateral growth of mesoblast along the mesoblast sac. The appearance of the nuclei of the epiblast and of the floor of the mesoblast sac is identical, and in mesoblast cells, which are continuous laterally with each of these tissues, there are here and there small dark nuclei, similar to the epiblast nuclei. These nuclei in mesoblast cells are not met with except in the immediate vicinity of the epiblast and the floor of the mesoblast sac. Under the blastoporic epiblast is a layer of mesoblast of limited lateral extent, which is sharply marked off from the overlying epiblast. This condition prevails in sections back of this point, but three sections an-

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

FIGS. 10-15. Cross-sections of an embryo of about the same age as Fig. 5, probably a little older. *bl.'po.*, blastopore; *can.n'ent.*, canalis neurentericus; *cd.d.*, chorda dorsalis; *e.bl.*, epiblast; *h.bl.*, hypoblast; *marg.bl.'po.*, margin of blastopore; *ms.bl.*, mesoblast; $\times 200$ diameters.

terior to Fig. 10 the separation cannot be made out (Fig. 12). In the third and fourth section forward (Figs. 12 and 13) several epiblast nuclei are distinguishable in the upper region of this mesoblast layer, otherwise its epiblast character has given place to that of mesoblast. The straggling nuclei indicate that the mesoblast underlying the mesoblast sac is mesoblast derived from the epiblast. Near the anterior end of the neurenteric canal this layer lies on each side of it, Fig. 14. While posteriorly the ventral mesoblast is separate from the epiblast of the floor of the mesoblast sac, it is continuous farther forward with lateral mesoblast which is plainly derived from the epiblast, as shown by the isolated epiblast nuclei above referred to. Hence, we are led to conclude that the lateral is derived mesoblast from two ectodermal sources in the sense just as explained. In Fig. 13 the tissue derived from the upper wall of the mesoblast sac in the middle line is the chorda, that from the upper wall on either side of the middle line is the upper layer of mesoblast (*ms'bl.ⁱ*), that from the lower wall of the mesoblast sac is the lower layer of mesoblast (*ms'bl.^{vi}*). In Fig. 14 where the mesoblast sac approaches the hypoblast, it divides the mesoblast from the lower wall of the mesoblast sac so that from here forward there is a lateral layer of lower mesoblast (*ms'bl.ⁱⁱ*) on each side. This condition can be traced forward for several sections. Ectoderm nuclei may be found here and there in the lateral mesoblast also, Figs. 10, 11, 12. In Fig. 11 is seen the line of juncture of chorda and epiblast just in front of the anterior lip of the blastopore. The chorda is well differentiated from the lateral mesoblast immediately in front of its place of origin (Fig. 12), but it becomes less sharply separated from it as one passes forward (Figs. 13, 14), and but a short distance forward it is quite indistinguishable from the lateral mesoblast. No doubt the differentiation of cells proceeds as a process from behind forward. There is no evidence that the chorda becomes separated from the lateral mesoblast by the fusion of the upper mesoblastic layer with the lower mesoblastic layer as in the *Chelonia*, Mitsu-kuri, 92, (Figs. 14, 15, 16). The significance of these two layers of mesoblast can be no other than that they are the upper and lower wall of a much compressed hollow pouch such as that described in the youngest embryo here figured (Figs. 1, 2 and 3). One must conclude that from the V-shaped depression seen at the entrance to the mesoblast sac in surface view (Fig. 5), and in section Fig. 10, a pouch highly compressed laterally has grown forward and laterally in a diagonal direction, that the upper wall of this pouch is derived from the epiblast at the sides and in front of the mesoblast sac and that the lower wall is formed by the spreading of a small knot of epiblast tissue in the floor of the meso-

blast sac laterally and forward, that the cavity of the pouch is obliterated to a mere crevice except in the median plane where it remains as the mesoblast sac, in its last condition, the canalis neurentericus, open ventrally where mesoblast and hypoblast come together.

DISCUSSION OF LITERATURE.

While the general process of gastrulation in *Phrynosoma* is similar to that in other reptiles, there are some striking differences. In *Hatteria* (Dendy, 99) there is a stage when the embryonic area very much resembles the cap-like elevation of the *Phrynosoma* embryo represented in Figs. 1-3. The ectoderm is similarly elevated and thickened, beneath is a cellular mass of hypoblast and on the posterior margin of the area is the blastopore, a depression of considerable breadth. From the vicinity of the blastopore, the mesoblast spreads laterally and the head process grows forward. This early growth of mesoblast, when the mesoblast sac is very shallow, exists in *Phrynosoma*. In *Hatteria*, however, the head fold either appears much earlier than in any of the lizards, or Dendy's figure is of a stage in which the blastopore has become closed with age. The lack of information as to further details of the development of the blastopore in *Hatteria* renders the above comparison of little value. Because of the work of Mitsukuri our knowledge of the embryology of the *Chelonia* is much more complete. In appearance, the blastoderm of *Clemmys Japonica* (Mitsukuri 94, Fig. 1, Pl. VI) as seen in surface view, is much the same as the older stages of *Phrynosoma* after the blastoderm has flattened on the yolk. None of Mitsukuri's figures suggest any elevation of the blastoderm. From his figures (Mitsukuri, 94, Figs. 9, 13, 15, Pl. VIII), it is very apparent that in *Clemmys* there is less cellular differentiation than in *Phrynosoma* at this early stage. The accumulation of yolk in and about the region of the primitive streak obscures the lateral spread of mesoblast. There are certainly no median or lateral cellular pockets at this or later stages. Mitsukuri describes a lateral growth of mesoblast from the primitive streak, but does not call attention to any evidence that it is a hollow evagination of the mesoblast sac. Similarly the growth of the head process anteriorly is *en masse* and gives no evidence of a ventral wall (Mitsukuri, 94, Figs. 15 and 16, Pl. VIII). Only in stages subsequent to the breaking through of the mesoblastic canal and the cellular differentiation of all three germ layers does Mitsukuri find evidence that the lateral mesoblast is an evaginated growth from between the hypoblast and chorda. There is a somatic and splanchnic mesoblast, the former continuous with the chorda, the latter con-

tinuous with the hypoblast which does not pass under the chorda. As we shall point out at another place, the condition here referred to may, and perhaps should, have a slightly different interpretation than that which Mitsukuri has put forward. The spread of mesoblast laterally from the primitive plate begins later in *Clemmys* than in *Phrynosoma* if we may assume that the blastopore appears at the same time in each case.

The early development of the Squamata differs but slightly in the two suborders. The Ophidia are like the Chelonia in having the cellular differentiation less marked than the Lacertilia. The shape of the embryonic area in the Ophidia is not clear from Hertwig's two figures of the earliest stages (Hertwig, 03, Fig. 415, *A* and *B*). The blastopore is more or less posterior to the embryonic area. It is a broad, posteriorly arched depression with no sharp angles. In slightly later stages (Hertwig, 03, Fig. 424, *A* and *B*) the embryonic area becomes narrow posteriorly, a condition not encountered in *Phrynosoma* at any time (Figs. 1 and 5). It is but slightly elevated in the Ophidia and consequently not sharply marked off from the extra embryonic area by a bench (*Phrynosoma*, Fig. 1). The blastopore of both moves, relatively speaking, into the embryonic area as ontogeny progresses. The mesoblast sac in the snake is clearly shown by Hertwig's figures (03, Figs. 427 and 428). It differs from *Phrynosoma* in having the epiblast and chorda anlage less thick and less compact and in having no specially marked lateral pockets from the blastopore. Cell islands in the subgerminal region are characteristic of the snake. At the time the mesoblast sac comes into communication with the subgerminal space, isolated groups of cells remain where the lower wall of the mesoblast sac and the hypoblast broke away, thus establishing the neurenteric canal (Hertwig, 03, Figs. 430, 431). While this condition probably exists in *Phrynosoma*, nothing of the kind has been observed in the stages thus far examined. Hertwig's figures of cross sections (Hertwig, 03, Figs. 443-447) represent a solid outgrowth of mesoblast from the lateral angles of the blastopore and at no place do they reveal a lamination of the mesoblast suggesting a separate origin for somatic and splanchnic layers.

The gastrulation of lizards as worked out by Wenckebach, 91, has nothing of striking contrast to the particular genus we are now studying. In *Lacerta* (Will, 95, Wenckebach, 91) and in *Platydictylus* (Will, 92, Taf. 6), there is not such a marked elevation of the embryonic area. Both have the mesoblast sac developed in a characteristic way, but conditions in the Gecko seem a little more closely allied to those of *Phrynosoma*. In a very early stage there is a much larger space between the

epiblast and hypoblast of the horned toad than in either of the forms just mentioned. This may account for the greater development of the lateral mesoblastic pouches of the horned toad. No figures of cross sections are given by either Wenckebach, or Will, that represent such a lateral extension of the mesoblast sac, yet it seems probable that they may exist. There is in the blastoderm of the Gecko a condition which corroborates the interpretation we have given above of the gastrulation in *Phrynosoma*, namely that there are two paired, and one median, hollow pouches derived from the sides and front of the mesoblast sac respectively. In Will's stage III (Fig. 48 Pl. 7) the mesoblast sac is very shallow, the head process is in evidence and presumably some lateral spreading of mesoblast. In stage IV (Fig 55, Pl. 9) the head process, through the extension of the mesoblast sac has become a long hollow blind sac, flattened considerably between epiblast and hypoblast. By the disintegration of the lower wall of the sac and of the underlying hypoblast (Fig. 57, Pl. 9) the mesoblast sac is placed in communication with the segmentation cavity. It is obvious that this median sac of the Gecko gastrula is homologous with the median sac of the *Phrynosoma* gastrula and that the condition shown in Figs. 2 and 3 is derived in the same manner as that in Will's Fig. 57, Pl. 9. But Will makes no mention of lateral pouches which are doubtless more conspicuous in *Phrynosoma* because as we have already pointed out, in the latter, there is no dorso-ventral compression. Will's explanation of the continuity of the chorda with the hypoblast in the anterior median region of the blastoderm is in full accord with conditions found in late gastrula stages of *Phrynosoma*. Will describes a similar median pouch in *Lacerta viridis* (Will, 95b, Fig. 36, Taf. 6).

In the early development of some lizards one does find a slight elevation of the posterior margin of the blastoderm (Will, 95a, Fig 36, Taf. 6, *L. viridis*; Will, 93), but in no sense comparable to the conditions in *Phrynosoma*. In other cases there is no suggestion of it (Weldon, 83, Wenckebach, 91, Strahl, 82). Conditions in the formation of the embryonic area and mesoblastic pouch in *Lacerta* are substantially the same as in the Gecko (Wenckebach, 91). There are some minor differences in the development of the chorda. The unpaired median invagination even at the very beginning forms a compressed triangular shaped cavity in the lizards, while it is much less compressed at first in *Phrynosoma*, becoming more and more compressed later. As in the Gecko, the genus *Lacerta* does not develop mesoblast by prominent hollow lateral pouches from the antero-lateral sides of the mesoblast sac. In fact, there is some confusion as to the exact origin of the mesoblast. Some authors (Will, 95b)

state that it arises, in part, from hypoblast budding before invagination and in part grows forward as a solid mass from the anterior region of the mesoblast sac in the middle of which the chorda becomes differentiated (Weldon, 83, Balfour, 79, Will, 95). Other investigators derive all the mesoblast from the sides of the chorda and from a lateral growth of the primitive plate after invagination has begun (Wenckebach, 91, Strahl, 83). There is a very close resemblance of the cross sections of *Phrynosoma* embryos in late gastrula stages, to cross sections of *Lacerta* and the Gecko figured by Will (95b, Figs. 43^a, 44^c, Taf. 7; 93, Figs. 59b, Taf. 10-Taf. 36 and of *Clemmys* figured by Mitsukuri, 92). Mitsukuri interpreted this condition as showing that the lateral mesoblast has been derived by a process of cell budding and an outgrowth from the edges of the chorda and the median margin of the hypoblast. Assuming that the absence of true hollow lateral sacs in *Lacerta* does not forbid one from regarding the lateral solid growth of mesoblast as having its origin in the primitive streak together with epiblast infolded on either side of the chorda anlage, one must regard the two layered mesoblast as having arisen in part from a posterior source and having migrated forward and also from the sides of the mesoblast sac as far forward as it may have extended. This agrees perfectly with the decrease in mesoblast as one passes forward as found by Mitsukuri in *Chelonia* (Mitsukuri, 92, Figs. 14, 15 and 16). But from this point of view, one cannot say that the mesoblast is derived from the hypoblast, nor is there any evidence in *Phrynosoma* that such is the case. The close connection of the hypoblast and splanchnic layer of the lateral mesoblast is a necessary consequence of the breaking away of the hypoblast and lower wall of the median invagination, or mesoblast sac, placing the chorda in contact with the hypoblast anteriorly and leaving the broken edges of splanchnic mesoblast and hypoblast on either side to grow together later as Mitsukuri describes when he says the point* moves to the point† (92, Fig. 16). According to the interpretation here given the somatic mesoblast will probably receive additions farther anterior than the splanchnic, the latter having a posterior source as its feeder and the former both a posterior source and possibly anteriorly from the chorda anlage. By comparing Fig. 13 with Fig. 15 we find the splanchnic mesoblast decreasing in lateral extent. In fact, there is practically no evidence in *Phrynosoma* that either the chorda or hypoblast contributes anything toward the origin of the mesoblast. Will, in his descriptions of the Gecko, has already presented the view here set forth, but Mitsukuri, 92, states that in *Chelonia* the hypoblast turns upward laterally to become the splanchnic layer of mesoblast and that the chorda contributes to the somatic mesoblast.

The egg of *Phrynosoma* stands in closer relation to the lower vertebrates than any other amniote in that the protoplasmic pole of the egg seems less encumbered with yolk, the blastoderm at least is so elevated that processes going on in it are quite as independent as in the Amphibian egg. We may say that the development of this iguanid is a link in the chain of evidence which supports the Weldon, 83,-Will, 93, theory of yolk cleavage wherein the lower layer tends toward the cleavage of the whole mass of the yolk as in the Axolotl, and the mollusc *Bythinia tentaculata*, figured by Weldon.

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² A complete bibliography of the embryology of Reptiles is found in Peter, 04.

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