THE DEVELOPMENT OF THE COTTON RAT, SIGMODON HISPIDUS TEXIANUS (Audubon and Bachman, 1853), THROUGH THE FIRST NINE AND ONE HALF DAYS OF GESTATION.

by

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THE DEVELOPMENT OF THE COTTON RAT, SIGMODON HISPIDUS TEXIANUS (Audubon and Bachman, 1853), THROUGH THE FIRST NINE AND ONE HALF DAYS OF GESTATION.

INTRODUCTION

The purpose of this paper is to present observations on early development of the cotton rat, Sigmodon hispidus texianus during the first 9½ days of gestation. Development of the cotton rat is compared with that of other rodents and relationships of Sigmodon are suggested. Embryonic development of the cotton rat has not previously been investigated. Meyer and Meyer (1944: plate 2, figures 8, 9, 10 and 11) published photographs of a freshly ovulated egg, three two-cell stages, two eight-cell stages and a blastocyst. These early stages had been flushed from the reproductive tubes and were described only briefly in the legends for the figures.

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MATERIALS AND METHODS

In order to estimate the age of embryos as accurately as possible, pairs of rats were placed together for mating only after the females were known to be in estrus. Estrus was detected by the vaginal smear method using the pipette technique (Snell, 1941); vaginal smears were stained with a trichrome mixture. Successful copulation was verified by noting the presence of a vaginal plug. Copulation was actually observed in only two instances. Other pairs that copulated did so when allowed to remain together for some previously determined period, such as two, three, four, eight or twelve hours. The age of embryos was calculated from the time of observed or estimated time of copulation. The female of a pair of rats that was left together for a specific period of time and that developed a vaginal plug, was arbitrarily assumed to have copulated in the middle of the period during which the animals had an opportunity to mate, and the age of embryos was estimated accordingly.

Females to be sacrificed were killed with ether or by pithing. The genital tubes were fixed immediately in Bouin's solution for 12 to 24 hours. After fixation the tissues were washed in running tap water and transferred to a solution of lithium carbonate to neutralize any remaining picric acid. Tissues were stained <u>in toto</u> in Harris' haematoxylin for 12 to 24 hours, treated with acidified 70 per cent alcohol to remove excess stain, and finally neutralized in basic 85

per cent alcohol. Sections were made by the paraffin method in the usual manner, and were cut at intervals of 10, 15, or 20 micra, according to the nature of the material. Smaller embryos were cut at intervals of 10 micra. Since it was not feasible to remove from the uterus embryos younger than 4½ days of age, sections of these were obtained by making longitudinal serial sections of the uteri containing them. Embryos 4½ days of age to 9½ days of age were easily located by localized uterine enlargements. These enlargements were separated and sectioned either parallel or transverse to the longitudinal axis of the uterus; a few 8½ day old and 9½ day old embryos were dissected from the uterus and fetal membranes and sectioned either parallel to the sagittal plane, or transverse to the crown-rump axis.

All measurements given were made of fixed or preserved material. Measurements of ova and cleavage stages do not include the pellucid membrane, and measurements of embryos older than 4½ days do not include the ectoplacental cone.

More than 300 animals were used in this study, and a total of 170 embryos were sectioned or dissected. A partial list of the eggs and embryos used in this study appears in table 1.

DESCRIPTION OF DEVELOPMENTAL STAGES

Ovarian Stages and Ovulation

Ovarian ova:- The development of the ovarian egg of Sigmodon is similar to that in other mammals. Primary follicles (figure 1, Plate I) are sparingly distributed in the cortex of the ovaries. A young primary follicle is approximately 68 micra in diameter and is made up of a single layer of follicle cells enclosing a primary oöcyte measuring approximately 40 micra in diameter. The nucleus of such an oöcyte is clear except for scattered chromatin granules which stain lightly with Harris' haematoxylin. Primary follicles grow in size as follicle cells increase in number.

As follicular growth continues, vacuoles or spaces appear among the follicle cells (figure 2, Plate I). These spaces coalesce and form an antrum, which marks the initiation of the secondary follicle. The follicle at this stage is approximately 280 micra in diameter. The oöcyte approximates 54 micra in diameter and is eccentrically situated. Its nucleus is clear and its chromatin granules stain lightly with Harris' haematoxylin.

When ovarian follicles are mature (figure 3, Plate I) they are found at the periphery of the ovary and approach 466 micra in diameter. They are distended by follicular liquid and bulge at the free surface of the ovary. As ovu-

lation approaches, the superficial part of the follicle is raised into a local elevation, the stigma.

The oöcyte is buried in a mass of follicular cells, the cumulus oöphorus, and is surrounded by a transparent membrane, the zona pellucida. Mature ovarian eggs measure approximately 82 micra along the main axis by 57 micra wide; the thickness of the zona pellucida is approximately five micra. The zona pellucida seems to be weak and flexible before ovulation (figure 4, Plate I; figure 1, Plate II). As ovulation approaches, cells of the cumulus oöphorus which adhere to the oöcyte constitute the corona radiata.

Ovulation:- Females that are sacrificed in estrus have some ova in the ovaries, whereas other ova are newly ovulated and are situated in the upper ends of the oviducts. Ovarian ova clearly show the process of the first maturation division; nuclei are in prophase (figure 4, Plate I) and metaphase (figure 1, Plate II). Some ovarian eggs float in the antral space in mature follicles. These ova (figure 2, Plate II) have undergone the first maturation division and the nuclei are in the metaphase stage of the second maturation division. Floating ova are approximately 66 micra wide and 73 micra along the main axis. A perivitelline space is appearing (figure 2, a, Plate II) and the first polar body is situated in it (figure 2, b, Plate II). Floating ova are further characterized by strands of adhering follicular cells, the corona radiata. Corona cells are large and vacuolized and

their nuclei stain darkly with Harris' Haematoxylin.

Newly ovulated eggs (figure 3, Plate II) still possess an adhering corona radiata. Nuclei of such ova are still in metaphase of the second maturation division.

Fertilization and Cleavage

One-cell stage:- All one-cell stages were found in the upper third of the oviducts. Some of the one-cell stages were newly ovulated ova (described above). Older one-cell stages (figure 4, Plate II) differ from the newly ovulated ova in that the cells of the corona radiata are dispersed and the ovum nucleus stains darkly with Harris' haematoxylin; the zona pellucida appears to be structurally tough and heavy. In all of the one-cell stages seen, only one polar body was observed and no pronuclei or cleavage spindles were observed. The more advanced one-cell stages are found 10 to 15 hours after copulation when the uterus is distended with fluid containing motile sperm. Although secondary polar bodies, pronuclear stages, and penetration of the ovum by sperm were not observed, dispersion of the corona radiata cells and the presence of sperm at this time indicate that fertilization takes place 10 to 15 hours after copulation.

Two, four, and eight-cell stages:- Two-cell cleavage stages (figure 1, Plate III) are situated in the upper third of the oviduct 24 hours after copulation; four-cell stages (figure 2, Plate III) travel as far as the middle third of

the oviducts by 36 hours. These stages are enclosed in a zona pellucida and often exhibit one polar body. Morulae of eight cells (figure 3, Plate III) are found in the antimesometrial crypts of the uterine tubes by 72 hours. Blastomeres vary in size; the largest blastomere is farthest from the uterine epithelium and is approximately 23 micra in diameter while the remaining blastomeres are approximately 15 micra in diameter.

Early blastocyst stage:- By 3½ days the early blastocyst, consisting of approximately 32 cells still enclosed in the zona pellucida, is formed. Externally the blastocyst is ovoid in shape approximating 70 micra along the main axis by 64 micra wide. The blastocoel is eccentrically situated toward the abembryonic zone. The blastocyst is composed of an inner cell mass and trophoblast. The trophoblast is composed of flattened cells which are situated around the periphery of the young embryo in a single-cell layer, enclosing the blastocoel and the inner cell mass. The inner cell mass is composed of eight to ten oval or spherical cells. The three blastocysts observed varied only slightly. Blastocysts of this stage are situated in the antimesometrial part of the uterus but have no particular orientation within it.

Endoderm Formation

Blastocysts of at least 96 cells form by three days, 16 hours (figures 1 and 2, Plate IV). At this time the blasto-

coel distends and the pellucid membrane disappears. Nine blastocysts of this stage of development were approximately 160 micra along the embryonic-abembryonic axis and 108 micra wide. Probably blastocysts are ovoid in shape in life, but in preparation for study many were distorted. With increase in size of the cavity of the blastocyst, the disc-shaped inner cell-mass becomes relatively less prominent.

The cells of the inner cell-mass, as seen in section, are elongate, about eight micra wide and 11 micra long. Cytoplasm is scanty and contains no distinct granules. Nuclei of these cells are large, vesicular structures making up about two-thirds of the bulk of the cells. Chromatin is granular, scattered and stains lightly with Harris' haematoxylin.

Trophoblastic cells are approximately six micra in thickness and 10 micra to 18 micra in diameter. However, cells of the trophoblast which touch the uterine epithelium are larger, approximately 10 micra in thickness and 19 micra in diameter (figure 1, c, Plate IV). Cytoplasm of all trophoblastic cells is scanty and refractory to staining, but cells which touch the uterine epithelium have relatively more cytoplasm. Nuclei of the trophoblastic cells are large and were frequently observed in various stages of mitosis.

Endoderm formation:- By three days and 16 hours, endoderm barely makes its appearance. A clearly formed layer of endoderm in the region of the inner cell-mass is not yet

discernible, but a few scattered cells, or in some blastocysts of this age, a strand of detached cells along the inner surface of the trophoblast signializes the first appearance of endoderm (figure 2, b, Plate IV). These endodermal cells seem to arise by delamination from the inner cell-mass and migrate around the inner surface of the trophoblast to form the distal endoderm. These observations of Sigmodon are similar to those of Heuser and Streeter (1929) for the pig. The cells of the trophoblast may be considered ectodermal, while the proximal endoderm and the ectodermal node form from the inner cell-mass. According to Heuser and Streeter (1929), development of the pig embryo, is similar to that of Sigmodon, as is also the development of Rattus norvegicus (Huber, 1915).

The trophoblastic cells above the inner cell-mass are not distinct by the fourth day. The trophoblastic cells seem to participate in the formation of the ectoplacental cone. Cell walls in the region (the forming ectoplacental cone) are syncytial and nuclei have chromatin granules which are scattered throughout the nucleoplasm or these granules are sometimes distributed around the periphery of nuclei. The chromatin granules stain lightly with Harris' haematoxylin. Cells of the forming ectoplacental cone join the inner cell-mass and dorsally cells of the ectoplacental cone mingle insensibly with the cells of the uterine tissue.

Differentiation of the Embryo and Embryonic Cyst

There is a marked differentiation of the inner cell-mass by the fourth day (figure 3, Plate IV). An embryonic ectodermal node is in the center of the inner cell-mass (figure 3, b, Plate IV) and embryonic ectoderm will form from this node. The nuclei of the cells of this node are oval and stain lighter than other cells of the inner cell-mass.

Nuclei of proximal endodermal cells (figure 3, c, Plate IV) are elongate in section and stain darkly with Harris' haematoxylin. Extraembryonic ectoderm (figure 3, a, Plate IV) forms from cells which are dorsal to the ectodermal node; these cells, which stain darkly with Harris' haematoxylin, have oval or irregularly shaped nuclei. As the cytoplasmic strands of the trophoblastic cells (figure 3, d, Plate IV) penetrate the uterine epithelium the trophoblastic cells swell and are partly obscured by epithelial and subepithelial cellular debris and pigment granules.

The inner cell-mass has grown deeply into the blastocoel by four days, four hours (figure 4, Plate IV). A proamniotic cavity appears by cavitation in the embryonic ectoderm (figure 2, a, Plate XI). It is circular and is approximately 66 micra in diameter. The "cone-shaped" ectoplacental cone is syncytial and contains many pigment granules (figure 4, a, Plate IV).

The egg cylinder, previously called the inner cell-mass, occupies most of the original blastocoel (figure 2, Plate V) in embryos 4½ days of age and the proximal endoderm seems almost to fuse with the distal endoderm.

The trophoblast which at this time is difficult to observe, is partly transformed into primary giant cells (figure 2, e, Plate V). These are three or four times the size of any of the surrounding cells. The cytoplasm is refractory to Harris' haematoxylin. Nuclei are approximately 19 micra in diameter. Chromatin is distributed in flocculent strands which stain darkly with Harris' haematoxylin, but nucleoplasm does not stain. Distal endodermal cells (figure 2, b, Plate V) appear to be scattered and to form an incomplete layer. Ventrally the endodermal cells are oval and enlarged in contrast to the smaller, flattened endodermal cells found elsewhere along the trophoblast. Distal endoderm is continuous with the proximal endoderm. The trophoblast surrounds the egg cylinder and terminates dorsally at the ectoplacental cone.

The egg cylinder is completely covered externally with a proximal, single-cell layer of endoderm (figure 2, c, Plate V). The cells of this layer tend to be cuboidal, with large nuclei. At the juncture between embryonic and extraembryonic ectoderm, the endodermal cells and nuclei become flattened and spindle-shaped. This type of cell covers the embryonic ectodermal region. At the antimesometrial (ventral) end of

the embryonic ectoderm the endodermal cells are large and oval, possessing large oval nuclei.

The embryonic ectodermal region of the egg cylinder is characterized by cells with round or oval nuclei and by cytoplasm that stains lightly with Harris' haematoxylin. Cytoplasm of cells in the extraembryonic ectodermal region of the egg cylinder, stains intensely (figure 2, a, Plate V) with Harris' haematoxylin and the nuclei appear elongate in section. Many of the nuclei are small and more of them show mitotic configurations than do cells of embryonic ectoderm.

The proamniotic cavity remains approximately 66 micra in diameter in embryos of 4½ days development.

The embryonic cyst:- The original proamniotic cavity is continuous with an ectoplacental cavity by the fifth day (figure 2, b, Plate V). The ectoplacental cavity, arising by cavitation, extends as a cleft through the extraembryonic ectoderm and slightly into the ectoplacental cone. This continuous cavity or cleft, composed of the combined proamniotic and ectoplacental cavities, is known as the chorioamniotic cavity. It is about 20 micra in width and approximately 170 micra in length.

Mesometrially there is a "cone-shaped" ectoplacental region (figure 1, a, Plate V). It is marked off from the egg cylinder by a constriction as it merges with the mesometrial extraembryonic ectodermal layer. The appearance of the ectoplacental syncytium is granular and cell boundaries are

indistinct. Nuclei of these cells are irregular in size. In some of these nuclei chromatin is not discernible, whereas others are filled with discernible chromatin granules or have one or two spheres which stain intensely with Harris' haematoxylin. Giant cells appear along the lateral borders of the ectoplacental cone and dorsad to the ectoplacental cone. These giant cells are numerous but most of them are relatively small, ranging between 10 micra and 12 micra in diameter. Except for their size these cells have the same appearance as the primary giant cells. They possibly arise from the cells of the ectoplacental cone.

The egg cylinder is readily divisible into a dorsal (mesometrial) elongate extraembryonic region (figure 1, Plate V) and a ventral embryonic ectodermal region. The dorsal part of the egg cylinder is surrounded by columnar endodermal cells (figure 1, c, Plate V). The nuclei of these cells are situated basally toward the ectoderm and cytoplasm at the distal region of these cells is vacuolated. The ventral region of the egg cylinder, knoblike in shape, is composed of embryonic ectoderm (figure 1, d, Plate V). The endodermal cells surrounding this region range in shape from cuboidal to flattened cuboidal.

The proximal endoderm of the egg cylinder approaches fusion with the distal endoderm distributed along Reichert's membrane (figure 1, f, Plate V). Any space that is evident between the endodermal layers represents the old blastocoel

(figure 1, e, Plate V). Antimesometrially (ventrally) the distal endodermal cells are flattened and are approximately eight micra long. However, at the extreme ventral end of the egg cylinder endodermal cells are larger measuring 10 micra long; they possess oval nuclei. Toward the ectoplacental cone the distal endodermal cells are approximately 10 micra long and are flattened in shape.

Twenty two embryonic cysts on the sixth day of development (figures 1 and 2, Plate VI) averaged 375 micra in length and 180 micra in width. The amnio-chorionic cavities of these embryos averaged approximately 268 micra in length and 84 micra in width.

Early appearance of the amnion and mesoderm:- Amniotic folds mark the juncture of the embryonic ectoderm with the extraembryonic ectoderm (figures 1 and 2, Plate VI). One fold (the posterior amniotic fold) protrudes more prominently into the chorio-amniotic cavity (figure 2, f, Plate VI) than the anterior amniotic fold. The pronounced protuberance is caused by a thickening of the ectoderm and the probable appearance of mesodermal cells (figure 2, d, Plate VI) delaminating from embryonic ectoderm. The embryonic ectoderm in the region of probable mesoderm appearance is somewhat syncytial in appearance. The development of possible mesoderm is observed only close to the median sagittal axis of the embryo and near the posterior end of the embryonic ectoderm.

The embryonic axis is established by the sixth day; it

lies in the shape of a "C" extending from the posterior to the anterior amniotic fold (figure 2, Plate VI). The embryonic region (figure 2, b, Plate VI) consists of the germinal disc which gives rise to the primitive shield (embryonic disc) and primitive streak.

Formation of fetal membranes:- Eighteen embryonic cysts observed in the seventh day of development average 685 micra in length and 465 micra in width. These embryonic cysts have almost doubled in size since the sixth day.

Besides undergoing rapid growth, these embryos are rapidly differentiating fetal membranes. At the sixth day, only amnion formation was indicated by the presence of the posterior and anterior amniotic folds, but the chorion, amnion, early allantoic bud, and the yolk sac are evident at seven days (figure 1, Plate VII).

As mesoderm is differentiated from the primitive streak, it moves laterally and caudally into the developing amniotic folds and eventually moves cephalad, penetrating between the embryonic ectoderm and endoderm and then extraembryonic ectoderm and endoderm (figure 3, Plate VI). Mesoderm moves into the region of the amniotic fold and in Mus this mesoderm is soon characterized by the appearance of small spaces which coalesce (Snell, 1941). This process of coalescence was not observed in Sigmodon, but a large, ring-shaped space is formed (figure 3, c, Plate VI). This space extends around the circumference of the embryonic cyst and is the definitive

exocoelom. The appearance of the exocoelom gives rise to the somatopleuric chorion (figure 1, c, Plate VII) and amnion (figure 1, f, Plate VII). Laterally the exocoelom is bounded by splanchnopleure; this is the yolk sac (figure 3, h, Plate VI; figure 1, d, Plate VII).

Just prior to the division of the original chorio-amniotic cavity into the chorionic cavity, exocoelom and amniotic cavity (figure 1, a, b, g, Plate VII) an amnio-chorionic pore is observed (figure 3, b, Plate VI). The amnio-chorionic pore is eventually closed and the exocoelom becomes a spacious cavity completely separating the chorion and amnion (figure 1, Plate VII).

The allantois is formed as a porous, mesodermal bud growing out from the posterior end of the primitive streak (figure 1, e, and figure 2, a, Plate VII). It does not have a cavity.

Four somite stage:- The embryo at 7½ days (figure 4, Plate VII) is characterized by four somites.

In the four-somite cotton rat embryo there is a head fold extending over a subcephalic pocket (figure 4, e, Plate VII). Formation of the head fold is accompanied by the appearance of the foregut and anterior intestinal portal (figure 4, f, Plate VII). In the mesenchyme between the gut endoderm and the ectoderm of the subcephalic pocket are located endothelial tubes of the developing heart (figure 4, g, Plate VII). Over the head fold and the raised part of the body,

the neural plate is differentiating into neural folds (figure 4, Plate VII). There are four or five pairs of somites (figure 4, d, Plate VII). A rod of closely arranged cells, the notochord, is situated in the median saggital plane of the embryo. Reichert's membrane and the distal endoderm are observed (figure 4, h, Plate VII). The allantoic stalk (figure 4, b, Plate VII) is elongate and extends toward the chorion (figure 3, c, Plate XI). The chorionic space (figure 3, b, Plate XI) is reduced to a small cavity as the chorion approaches the placental region.

Seven somite stage:- A comparison of the seven-somite embryo with the four-somite embryo well illustrates the rapid development which takes place within a one-day interval.

The circulatory system shows extensive differentiation and is assumed to be functional since blood cells are observed throughout the embryonic and extraembryonic circulation. The embryonic circulatory system consists of a single tubular, flexed heart (figures 2 and 3, Plate X), a ventral aorta w which immediately divides into the first and second aortic arches (figure 4, b, Plate X). The extraembryonic circulatory organs consist of vitelline vessels (figure 3, a, Plate XI) which course to and from the yolk sac through the margin of the midgut (figure 3, b, Plate XI). Blood cells in the blood vessels are numerous (figure 2, b, Plate X). The bulbar, ventricular and atrial regions of the heart can be distinguished (figure 2, b, c, and figure 3, a, Plate X). The cardiac

coelom is established.

In the nervous system, the several vesicles of the brain, the telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon can now be recognized. The vesicles are open at the dorsal surfaces (figures 1 and 3, Plate X). Optic vesicles have evaginated from the diencephalon (figure 4, a, Plate X) and the otic placodes have appeared in the somatic ectoderm lateral to the myelencephalon and are invaginating (figure 1, d, Plate X). The neural tube is open (figure 4, Plate XI). Several cranial ganglia are appearing; the semilunar and the geniculate ganglia.

In the four-somite stage (7½ days) the mid-gut was an open space and there was no hind-gut (figure 4, Plate VII). By 8½ days the gut is complete except at a restricted mid-gut region where the splanchnopleure of the gut becomes continuous with the true yolk sac (figures 3 and 4, Plate XI). The foregut is differentiated to form a pharynx (figures 1 and 4, Plate X) with visceral pouches which are separated from the outside by closing membranes (figures 1, 3, Plate X). The pharynx is separated from the stomadaeum (figure 1, f, Plate X) by an oral plate which is perforate. The allantois is fused to the chorion (figure 3, c, Plate VII).

Flexure and torsion:- Flexure and torsion of the embryo takes place within the 7½ and 8½ day interval. Rotation is toward the left side of the embryo. A cephalic flexure brings the maxillary region of the head into adult relationship with

the mandibular arch (figure 3, b, Plate X). There is also a caudal flexure at the posterior end of the embryo (figure 1, Plate XI). At six days the embryo is "C-shaped" with the ventral surface on the convex side of the "C" (figures 1 and 2, Plate VI). By 7½ days the embryo is almost flat (figure 4, Plate VII) and with the ventral mid-gut region outside the embryo. By 8½ days the external form of the embryo, owing to torsion and flexure, is an "S-shaped" spiral with the ventral surface enclosed within the embryo (figure 1, Plate XI).

IMPLANTATION AND PLACENTATION

In order to present a concise and clear discussion of implantation and placentation, this subject is presented in a separate discussion beginning with the inception of implantation through 9½ days.

The blastocyst is characterized by a superficial attachment to the uterine epithelium by three days and 16 hours (figures 1 and 2, Plate IV). The uterine epithelium undergoes some degeneration in the region of this contact: Pigment granules are scattered in the region of this contact; epithelial cell walls are indistinct and the position of the nuclei of these cells gives the epithelium a pseudostratified appearance (figure 2, Plate IV); there are eroded concavities in the uterine epithelium occupied by the cells of the trophoblast (figure 1, Plate IV). The first attachment

of the uterine epithelium seems to be easily broken since some of the blastocysts appear to have been detached, showing fragmented tissue where the blastocysts would normally be attached to the uterine epithelium. Cytoplasmic strands of the enlarged trophoblast cells (figure 3, d, Plate IV) are penetrating the uterine epithelium by the fourth day. In these regions of penetration there are pigment granules and cellular debris arising from degenerating epithelial and subepithelial cells and their nuclei. By four days the cells of the endometrium are undergoing a decidual reaction, as a result of which they become large and epithelioid. These cells are usually vacuolated and the cell walls are obscure. Their cytoplasm stains lightly and the nuclei are large and clear except for chromatin granules.

The decidual reaction (described above) reaches about half way through the endometrium by four days, four hours (figure 1, Plate IX). There is a "cordlike" distribution of decidual cells (figure 2, Plate IX) accentuated by flattened sinuses (figure 2, c, Plate IX) which radiate ventrally and diagonally from the embryo and uterine lumen toward the ventral periphery of the uterus. Three zones are characteristic of the decidua capsularis. In the inner zone (figure 2, b, Plate IX), decidual cells are small and closely packed together, but in the middle zone (figure 2, d, Plate IX), decidual cells are vacuolated, large and loosely arranged. In the distal zone (figure 2, e, Plate IX) the decidual cells are small

and mingle indistinctly with the unchanged endometrial cells. The unchanged endometrial cells, peripheral to the distal zone, are polygonal in shape and give the tissue a loose texture. A decidual cavity has appeared (note the cavity around the embryo in figure 2, Plate IX). Primary giant cells which are located in this cavity erode decidual tissue and enlarge the decidual cavity. Blood sinuses are more prominent than at four days, and maternal blood cells are found around the embryo (note the small dark spots below the embryo in figure 2, Plate IX). The uterine lumen closes around each implantation site (figures 1 and 2, Plate IX). These thickened uterine implantation sites are apparent upon gross inspection of the uterine tubes when they are excised. These sites arise as the decidual cells undergo mitosis and growth. This growth begins with the inner decidual zone and progresses toward the muscularis of the uterus.

By four days, four hours two decidual membranes make their appearance. The decidua capsularis reaches from the decidual cavity laterally and ventrally (figure 1, a, Plate IX). The decidua basalis appears between the ectoplacental cone and the uterine lumen (figure 1, c, Plate IX). By $4\frac{1}{2}$ days the decidua capsularis reaches almost to the muscularis in the ventral region of the uterus and the decidua basalis reaches almost to the dorsal muscularis.

By the fifth day the decidual capsularis reaches to the muscularis and the decidua basalis extends into the muscularis.

Primary giant cells may be seen in the decidual cavity.

The decidua basalis is extensive by the sixth day and the decidua capsularis has reached its maximum development. As the decidual growth moves outward, the inner decidual regions are attacked by giant cells which are moving outward from the trophoblast. The process is apparent by the sixth day; the decidual cavity enlarges as the giant cells continue to attack the decidua capsularis (figure 1, Plate VI). Decidual cells in the region of attack are degenerating as evidenced by the presence of pigment granules, pycnotic nuclei and disappearing cytoplasm.

By the seventh day the decidual transformation reaches the muscularis on all sides of the embryo (figure 4, Plate IX). The decidua basalis (figure 4, a, Plate IX) is extensive and penetrates the dorsal muscularis. Its lateral margins are extensively coursed by large blood sinuses (figure 4, Plate IX). The decidua capsularis (figure 4, c, Plate IX) is regressing; giant cells are particularly active in the decidua capsularis and blood sinuses are lacking in it. Maternal blood sinuses are in contact with Reichert's membrane (figures 3 and 4, Plate VI; figures 1 and 2, Plate VII). Uterine glands are not discernible.

By 8½ days the decidua basalis is massive, permeated by blood sinuses and appears to be "spongy" (figure 3, g, Plate VII). By comparison with the basalis, the capsularis is thin (figure 3, b, Plate VII). The decidua capsularis is

detaching from the muscularis making the uterine lumen continuous again (figure 3, a, Plate VII), but opposite to the original lumen. The ectoplacental cone is permeated with small blood sinuses and is fused with the decidua basalis. The chorion and allantois are also fused to the decidua basalis. Thus the embryo has contributed the ectoplacental cone, the chorion and the allantois to the placenta. When the chorion fuses to the ectoplacental cone the yolk sac fuses to the peripheral margin of the definitive placenta (figure 3, Plate VII). Trophoblastic vessels are formed (figure 3, e, Plate VII) and maternal labyrinths (figure 3, f, Plate VII) are numerous.

ANGIOGENESIS AND PLACENTAL RELATIONSHIPS

From 7½ days through 9½ days the yolk sac is the hemopoetic organ of the embryo. At 7½ days scattered blood islands appear in the splanchnic mesoderm of the yolk sac (figure 1, Plate VIII). The cells in these islands are irregular in shape and have large, distinct nuclei. By 8½ days the entire yolk sac mesoderm is undergoing angiogenesis (figure 4, a, Plate XI). Some cells are differentiated into endothelial cells while others become hemoblasts. The mesodermal wall of the yolk sac is now an "area vasculosa." Hemoblasts are found in abundance in the blood vessels (figures 1, 2, and 3, Plate X) of the embryo, but they

are negligible or lacking in the fetal placental vessels (figure 3, Plate VII). By 9½ days hemoblasts are found in abundance in the fetal placental blood vessels and blood vessels of the embryo and are in all stages of mitosis (figure 2, b, Plate VIII).

The yolk-sac and Reichert's membrane are the only structures through which exchange between the embryo and the mother occur until 9½ days. By 9½ days fetal blood is circulating in the trophoblastic vessels (figure 2, c, Plate VIII), and the placenta assumes its function. At this time scattered hemoblasts are circulating through the spongy allantois indicating the appearance of an umbilical circulation. No umbilical vessels are identifiable, however, and the allantois remains porous.

Sigmodon is characterized by a chorio-allantoic placenta. The chorion is fused to the ectoplacental cone and is vascularized by allantoic mesoderm. At 8½ days the placenta is characterized by a labyrinthine, hemo-chorial condition (figure 3, Plate VII and figure 2, Plate VIII), but by 9½ days the placenta is approaching a hemo-endothelial condition (figure 2, d, Plate VIII).

Table 1

Summary of Ova and Embryos Available for this Study.

Embryonic Age	Developmental Stage	Number of ova or Embryos	Figures	Average Measurements Width	Main Axis
	Ovarian (early and late prophase and metaphase	12	4, Plate I; 1, Plate II	57u	82u
	Ovarian (Free floating)	12	2, Plate II	66u	73u
	Tubal (Newly ovulated with coronal cells)	4	3, Plate II	50u	50u
16 hr.	Tubal (one cell)	5	4, Plate II	50u	50u
24 hr.	Tubal (two cell)	3	1, Plate III	60u	60u
36 Hr.	Tubal (three-four cells)	4	2, Plate III	60u	60u
3 days	Uterine (eight cell morula)	3	3, Plate III	60u	70u
3 1/2 days	Blastocyst (32 cells)	3	4, Plate III	64u	70u
3 days 16 hrs.	Blastocyst (attachment)	9	1, 2, Plate IV	108u	160u
4 days	Implantation	2	3, Plate IV	120u	160u
4 days 4 hrs.	Implantation	11	4, Plate IV	85u	120u
4 1/2 days	Early egg cylinder	6	2, Plate V	120u	220u
5 days	Embryonic cyst	9	1, Plate V	140u	345u
6 days	Amniotic fold	22	1, 2, Plate VI	175u	345u
7 days	Chorion, Allantois yolk sac	18	3, 4, Plate VI 1, 2, Plate VII	465u	685u
7 1/2 days	Four somite	9	4, Plate VII	960u	1035u

DISCUSSION

The Problem of Calculation of Embryonic Age

In order to establish the age of embryos, it is necessary to know when copulation, ovulation and fertilization occur. Observations of Clark (1936) and Meyer and Meyer (1944) indicate that ovulation occurs in late estrus. My observations differ in that I found newly ovulated ova when I sacrificed females at the earliest indication of estrus as well as ova in mature ovarian follicles which would be ovulated later. Meyer and Meyer (1944) state that the duration of estrus in Sigmodon varies from one to 12 days (average 5.6 days) where the animals are exposed to disturbing conditions and one to four days (average 1.7 days) where the animals are situated in a quiet environment. When estrus averages 1.7 days, ovulation can be expected to continue through as much as 24 hours or more. However, I placed a male with a female as soon as the female gave evidence of estrus, since Bowman (1951) reported that in Rattus the incidence of fertilization was markedly lowered when copulation occurred late in estrus. The time of copulation can be, of course, under the control of the investigator and after I placed a male with a female I either observed copulation, or allowed selected intervals of time to elapse. On the occasions when I observed copulation, it was noted that the male would mount the female time after time. In Sigmodon fertilization

probably occurs about 10 to 15 hours after copulation. While not conclusively establishing that fertilization had occurred, one-cell stages 10 to 15 hours after copulation had lost their corona radiata cells and the uterine tubes and oviducts were distended with fluid containing motile sperm. Odor and Blandau (1951) in their studies of fertilization and the first segmentation division in rat ova observed that a number of ova were penetrated by sperm 11 to 13 hours after the onset of heat (upon determination of the onset of heat females were immediately placed with males). It is indeed probable that in Sigmodon fertilization occurs 10 to 15 hours after copulation.

In several instances I observed copulation which was judged unsuccessful because there was no vaginal plug and continued observation showed that pregnancy did not occur. Females were carefully examined throughout the investigation to be sure that a vaginal plug was formed and when a vaginal plug did not form male mates were replaced with others. Alternating several males with a female in estrus was successfully used by Wright (1948) in his work with the weasel.

There is variation in the developmental stage reached by embryos of Sigmodon after a given interval of time, arising from variations in time of ovulation, fertilization, rate of travel of the ova down the oviducts and rate of implantation.

Not only does development among embryos differ, but also

growth after parturition. I have observed that entire litters of Sigmodon characteristically grow slowly, while other entire litters characteristically grow relatively rapidly. The slower development after parturition can almost always be correlated with excitable animals, whereas the relatively rapid development after parturition usually is correlated with less excitable animals. MacArthur (1944) in his work with mice, selected for body size alone and found these animals differed unexpectedly in many other characters and traits such as behavior, hair color, relative length of the appendages and litter size. He stated that the large "race" has certain distinguishing coat colors, is more docile and inactive, has comparatively shorter ears, feet and tail, and bears many more young per litter. He suggests that large litters result from superovulation in the large race and that this is regulated by the gonadotropic hormones of the anterior pituitary. Finally he suggests that differences between "races" of mice, such as size of litter, or length of appendages are not determined by special fertility factors or ear-length genes, but in great part by the same common or general multiple size or growth rate factors that control size of body. Regarding the embryological basis of size inheritance in the rabbit, Gregory and Castile (1931) observed that development of eggs of large adult animals was more rapid than those of small, adult rabbits. They suggested that a greater richness of the nucleus in the "-SH group" may be

responsible for such rapid growth, but whether such compounds occur as genes located in particular chromosomes is unknown. The idea of the "-SH group" is based on Hammett's postulate that "The -SH group is the essential stimulus to growth by increase in cell number." Hammett (1930) stated that this chemical group is naturally concentrated in regions where growth by cell proliferation is taking place, and that the process is inhibited when the -SH group is removed. The factors underlying differences in rate of development among embryos are obscure and seem to be complex. In Sigmodon, however, although degree of excitability cannot be readily measured, it can be subjectively observed when handling the animals and it seems that degree of excitability of Sigmodon is familial and that selection for a low degree of excitability may be possible. At the same time, animals would be obtained that develop relatively rapidly. This type of selection has not been practiced with Sigmodon and variation in embryonic developmental rates arising from the causes just discussed may be expected.

PROBLEMS CONCERNING THE DEVELOPMENTAL STAGES AND RELATIONSHIPS

Variation in ovulation time:- Ovulation in Sigmodon occurs independently of copulation. Meiotic divisions and ovulation were observed in ovaries and oviducts of females which were sacrificed as soon as the vaginal smears showed

early estrus. The females were not placed with males, yet material representing preovulation and ovulation stages was obtained. Although I have established that ovulation occurs independently of copulation, it cannot be stated that ovulation occurs at any specified time in the estrus cycle. All stages of meiosis and ovulation are represented when the female rat is sacrificed in early estrus. Some of the ova have already ovulated, others are floating free in the ovarian follicle with the first polar body in evidence and others are in the prophase stage of meiosis (figure 4, Plate I; figures 1, 2, 3, Plate II). It is thus evident that one must consider an "ovulation period" which can extend from late proestrus to late estrus and represents a variation of one to 24 hours.

My observations indicate that the nucleus of the ovum remains in metaphase of the second meiotic division until fertilization occurs. The ovum nucleus is in metaphase of the second maturation division in the floating ovarian ovum (figure 2, Plate II) and the ovum nucleus is still in metaphase in the newly ovulated ovum (figure 3, Plate II). Both of these ova figured on Plate II came from females which had not been placed with males. Unfortunately I have no one-cell stages which positively show fertilization taking place and the subsequent changes which take place in the nucleus.

Change in texture of the zona pellucida:- I described the zona pellucida as being structurally weak and flexible in

the ovary but structurally heavy and tough after ovulation. This change in the zona pellucida is not related to fertilization because newly ovulated eggs which are not fertilized show this change (figure 3, Plate II). This change probably is related to protection of the early embryo from mechanical injury in the oviduct. Huber (1915) suggests this as a possibility in Rattus. It is remarkable that the shape of the early embryo is almost constant and well preserved until early attachment stages of the young blastocyst. I have observed that the zona pellucida does not thicken and consequently a space between the zona pellucida and the vitelline membrane would be due to shrinkage of the vitellus as yolk and cytoplasm are used in metabolism. Squier (1932) in studies of the guinea pig and Venable (1946) in observations of the hamster have described a similar shrinkage of the vitellus.

Variation in cell size in the eight-cell embryo:- I described a variation in size of the blastomeres of the embryo of Sigmodon at the eight-cell stage. One of the blastomeres approximated 23 micra in diameter, while the remaining blastomeres were approximately 15 micra in diameter. This seems to indicate that already cells are differentiated into two generalized groups: The large cell is cleaving slower and will give rise to the inner cell mass and the embryo, whereas the smaller cells are cleaving more rapidly and will give rise to the trophoblast. Heuser and Streeter (1929) published similar observations concerning the early

development of the pig.

The location of the implantation site and implantation:- The region in the uterus at which implantation takes place, in Sigmodon, apparently varies from the hamster, in particular. Implantation does occur on the antimesometrial side of the uterine lumen in Sigmodon, but not in close proximity to "implantation cups" described by Graves (1945) for Cricetus or not necessarily in antimesometrial grooves as described by Ward (1948) in Cricetus. In Sigmodon implantation may take place in uterine grooves or along a flat, straight uterine wall. At the point, where ever it may be, of first attachment the nuclei of uterine epithelial cells are dislocated in varying positions. The resulting condition was described by Krehbiel (1937) in the rat as "pseudostratified". In Sigmodon this "pseudostratified" appearance is initially evident only in the vicinity where the enlarged trophoblast cells contact the uterine epithelium (figures 1 and 2, Plate IV). Ward (1948) observed a similar situation in Cricetus.

Embryos of Sigmodon, like all rodent embryos so far as known (Mossman, 1937) make their first attachment at the abembryonic pole of the embryo. Like Mus, Cricetus, and Rattus, Sigmodon is characterized by a partly interstitial implantation. Implantation in Sciurus is superficial and eccentric. Dipodomys and Geomys are characterized by a partly interstitial implantation (Nielson, 1940; Mossman and Hishaw, 1940). Cavia implants completely interstitially

(Mossman, 1937). I am of the opinion (as is also Mossman, 1937) that in Rodentia there is a trend of evolution from the eccentric and superficial type of implantation to the completely interstitial type of implantation.

Inversion of the inner cell mass and the yolk sac:- The phenomenon of inversion characterizes embryonic development of Sigmodon. Inversion is accomplished in Sigmodon as the inner cell mass grows down into the blastocoel (figure 3, Plate IV) and carries the proximal endoderm and embryonic ectoderm deep into the segmentation cavity until proximal endoderm almost fused with distal endoderm (figure 4, Plate IV). The phenomenon of inversion is illustrated by Snell (1941) in a diagram comparing the thirteen-lined squirrel (Citellus) and the mouse (Mus).

Inversion of the yolk sac also occurs. The original trophoblast, lined with endoderm is an ephemeral yolk sac, whereas the splanchnopleure forming the periphery of the exocoelom is the true yolk sac (figures 3 and 4, Plate VI; figure 1, Plate VII). The terms give the impression that these two yolk sac tissues are different. Study of the ontogeny of the yolk sac in the Muridae (which show inversion) and Sciuridae (which show little inversion) reveals, however, that the endoderm of the ephemeral yolk sac and the true yolk sac are the same, but inversion places them in different topographic relationships. Furthermore, mesoderm penetrates part way between the distal endoderm and trophectoderm in the

Sciuridae, but does not in Sigmodon. Finally, as the name implies, the ephemeral yolk sac is transitory, but my observations did not elucidate the fate of the ephemeral yolk sac since the study was not continued for a sufficient interval of the developmental period.

In addition to the terms ephemeral and true yolk sac, "bilaminar yolk sac" and "splanchnopleure yolk sac" are used. Mossman (1937) used the term bilaminar yolk sac rather than ephemeral yolk sac and the term splanchnopleure yolk sac rather than true yolk sac.

In Sigmodon, as was mentioned above, the fate of the bilaminar or ephemeral yolk sac has not been determined, but the trophectoderm has completely participated in giant cell formation by six days and the distal endoderm is discernible until 9½ days. A bilaminar yolk sac has been observed in Citellus (Mossman and Weisfeldt, 1939) and consists of ectoderm and endoderm in early embryonic stages. It persists the full term. In Dipodomys and Geomys (Mossman, 1937) there is a bilaminar yolk sac, but it persists only until mid-term. The bilaminar yolk sac disappears at about the time of the appearance of the neural groove in Mus, Rattus and Cricetus persisting only on the placental surface (Mossman, 1937).

The splanchnopleuric yolk sac in Sciurus is vascular, incompletely inverted, small and nonvillous (Mossman, 1937). In Dipodomys (Nielson, 1940), Geomys (Mossman and Hishaw, 1940), Mus, Rattus, Cricetus and Cavia (Mossman, 1937) the splanchno-

pleuric yolk sac is vascular, completely inverted and is permanent. It is large, attached to the fetal surface of the placenta and is villous on the surface near the placenta. The development of Sigmodon was not continued for a sufficient interval of the developmental period to determine the entire fate of the true yolk sac, but the yolk sac is vascular, completely inverted and large. It is attached to the fetal surface of the placenta.

Beginning with the primitive rodents and climaxing with the specialized rodents there is an evolutionary trend toward an earlier, more complete inversion. Mossman (1937) concurs with this opinion. In the suborder Sciuromorpha this evolutionary trend is apparent in a progression from the primitive slightly inverted situation to the completely inverted situation (see above). A completely inverted situation is observed in the Hystricomorpha and the Myomorpha.

The significance of the phenomenon of inversion is unknown. Snell (1941) states that a "consequence of inversion of the germ layers is the production of a very compact form of early development." It can be inferred that the need in small rodents for special utilization of all available space may be correlated with the occurrence of this characteristic. I have observed that inversion occurs predominantly in relatively small rodents, which have large litters and which have relatively short gestation periods. The small size of the animal and the large size of the litter limit the space available for embryonic development and the shortness of the

gestation period allows for a relatively temporary expedient such as inversion.

Another interesting observation is that the phenomenon of inversion occurs in the Lagomorpha. Taxonomists, including paleontologists, have shown that the rabbits are distinct from the Rodentia, so and accord each group ordinal rank. These distinctive characteristics so separate the rabbits from the rodents that it is possible that the phenomenon of inversion appearing in Lagomorpha is an indication of parallel or convergent evolution. However, it is generally considered that the morphogenesis of fetal membranes is conservative and that morphogenesis of the fetal membranes results in slight differences within orders and between families. Since inversion of the germ layers modifies morphogenesis of the amnion, chorion and yolk sac, it may be suggested that inversion in Lagomorpha and Rodentia indicates a true genetic relationship between the two orders, rather than parallel or convergent evolution.

The function of the allantois:- In Sigmodon the allantois is a porous stalk of mesoderm reaching from the posterior end of the embryo to the chorion, and the stalk has no cavity. Eventually the umbilical vessels course through this stalk, the beginning of which is seen at 9½ days. It must be emphasized that the allantois has no excretory function in Sigmodon, but functions entirely in association with the formation and the function of the umbilical and

placental circulation. Mossman (1937) indicates that the primary function of the allantois in the Rodentia is vascular. Snell (1941) shows that this vascular function of the allantois is also true for Mus.

In Sciurus there is a small permanent allantoic vesicle (Mossman, 1937), but in rodents such as Dipodomys, Geomys, Mus, Rattus, Cricetus and Cavia there is no allantoic vesicle. The allantois in these animals is a spongy cord carrying the umbilical blood vessels.

The appearance of, and the changes in, the decidua:- The decidua capsularis was shown to reach its maximum extent at six days in embryos of Sigmodon. After this age the decidua capsularis regresses. Observations of various investigators indicate that three factors are responsible for the regression. Ward (1948) suggested that the attack of giant cells along the lateral and antimesometrial margins of the decidua capsularis is responsible for regression of the decidua capsularis. This phenomenon reaches its height of activity at six days in Sigmodon. Another factor, which Mossman (1937) describes is that flow of blood through the decidua capsularis is cut off and in Sigmodon the flow of blood is largely cut off by seven days. A third factor suggested by Mossman (1937) is the marked growth of the embryonic cyst which seems to exert considerable pressure on the decidua capsularis and which stretches the membrane. In Sigmodon there is marked growth of the embryonic cyst from five to

seven days. Each of these factors seem to have a part in the regression of the decidua capsularis in Sigmodon.

Mossman (1937) suggests that one of the evolutionary trends in the Rodentia is toward a more complete and more persistent decidua capsularis. In a sense this is true: In Sciurus the capsularis is incomplete (Mossman, 1937); Dipodomys has a complete capsularis early in development, but the capsularis disappears about mid-term (Nielson, 1940); in Geomys there is a complete capsularis early in development, but the capsularis is absent by mid-term (Mossman and Hishaw, 1940); Mus and Cricetus have a complete capsularis, but it disappears by limb bud stages (Mossman, 1937); there is a complete capsularis in Cavia which disappears in early fetal stages (Mossman, 1937); Sigmodon has a complete capsularis, but it is regressing by 9½ days. However, in Rodentia (excepting the primitive rodents such as Sciurus) I can see no indication that the capsularis is more persistent in the specialized rodents than it is in the more primitive rodents. In one of the most specialized rodents (Cavia), the capsularis disappears in early fetal stages. This type of development of the capsularis in Cavia appears to me not to be an advance and may be a regression.

The mechanics of torsion:- I have no material through the 7½ day to 8½ day interval of development to indicate how torsion occurs. However, Snell (1941) and Graves (1945) indicate that the twisting of the body is rapid and occurs in

two or three hours. Long and Burlingame (1938) and Graves (1945) further explain the mechanics of the twisting by saying that the early attachment of the allantoic stalk to the placenta and the coiling of the tail to the right of the head around the proximal end of the allantoic stalk govern the rotation of the embryo.

Placental relationships:- The character of the erythrocytes (hemoblasts), nucleated or non-nucleated, permits one to distinguish between fetal and placental vessels in Sigmodon. Graves (1945) made similar observations in the hamster. Further, maternal blood cells are smaller than the fetal blood cells in Sigmodon, as well as other rodents.

Mossman and Weisfeldt (1939, figure 41, Plate 8) illustrate placental relationships in the squirrels which are similar to the cotton rat at 9½ days, except that Sigmodon has smaller trophoblastic vessels and has not reached the degree of differentiation that is shown for the 20 mm. squirrel fetus. All of the fetal blood cells in Sigmodon are nucleated by 9½ days, whereas some of the squirrel fetal blood cells are non-nucleated; Sigmodon has some trophoblastic vessels that are lined with a thin wall approaching one-cell in thickness indicating that the true placental situation is hemo-endothelial, when differentiation is complete, whereas in Citellus the trophoblastic walls never approach this thinness and the placental situation is hemo-chorial.

The hemo-chorial placenta in Citellus represents a primitive

situation in the Sciuromorpha. The evolutionary trend is toward a hemo-endothelial placenta in Sciuromorpha and the hemo-endothelial placenta characterizes Myomorpha and Hystricomorpha.

In Sigmodon the placenta is formed by a fusion of the decidua basalis with the ectoplacental cone, the fusion of the chorion to the ectoplacental cone and the fusion of the allantoic stalk to the chorion. Although the allantois is never a vesicle in Sigmodon the allantois does vacularize the chorion and the placenta may rightly be considered chorio-allantoic.

Amniogenesis:- In Sigmodon the chorio-amniotic cavity forms in the inner cell mass by the process of cavitation. Modified amniotic folds then subdivide this cavity into chorionic, amniotic cavities and exocoelom. The same process is observed in Mus (Snell, 1941), Cricetus (Graves, 1945) and Rattus (Long and Burlingame, 1938). A primitive form of amniogenesis takes place in Sciuridae and occurs by simple folding as in reptiles and birds (Mossman and Weisfeldt, 1939). In Dipodomys amniogenesis takes place by a modified type of folding and a small closed chorionic cavity is folded off at the same time (Nielson, 1940). In Geomys there are small amniotic folds with secondary chorionic folds which never close and leave an open chorionic rudiment (Mossman and Hishaw, 1940). Amniogenesis in Cavia occurs in two regions; cavitation occurs in the embryonic region of the

inner cell-mass, and cavitation occurs in the extraembryonic region of the inner cell-mass (Mossman, 1937).

The evolutionary trend of amniogenesis is remarkably distinct. The primitive form of amniogenesis, is observed in the Sciuroidea, whereas in the Geomyoidea there is an intermediate form of amniogenesis; Geomys displays a more primitive form of amniogenesis than Dipodomys. The next advance is found in the Muroidea and the most specialized form of amniogenesis is observed in the Hystricomorpha.

Shape of the ectoplacental cone:- From the primitive rodents to the most specialized there is an evolutionary trend from a "giant cell ring" or "Träeger" to a "cone-shaped" ectoplacental cone. Mossman (1937) published similar observations for Rodentia. Graves (1945) observed that Cricetus is characterized by a "mushroom-shaped" ectoplacental cone. The cricetid "mushroom-shaped" ectoplacental cone appears to be intermediate between a "giant cell ring" and the "cone-shaped" ectoplacental cone. Graves'(1945) opinion was that the Cricetus is a comparatively close relative of the geomyid rodents. Sigmodon possesses a "cone-shaped" ectoplacental cone, which characteristic it shares with Microtus (Sansom, 1922, specifically stated that the ectoplacental cone in Microtus is "cone-shaped"). Furthermore Sigmodon shares this characteristic with Mus, Rattus and Cavia.

COMPARATIVE DEVELOPMENTAL RATES AMONG RODENTS

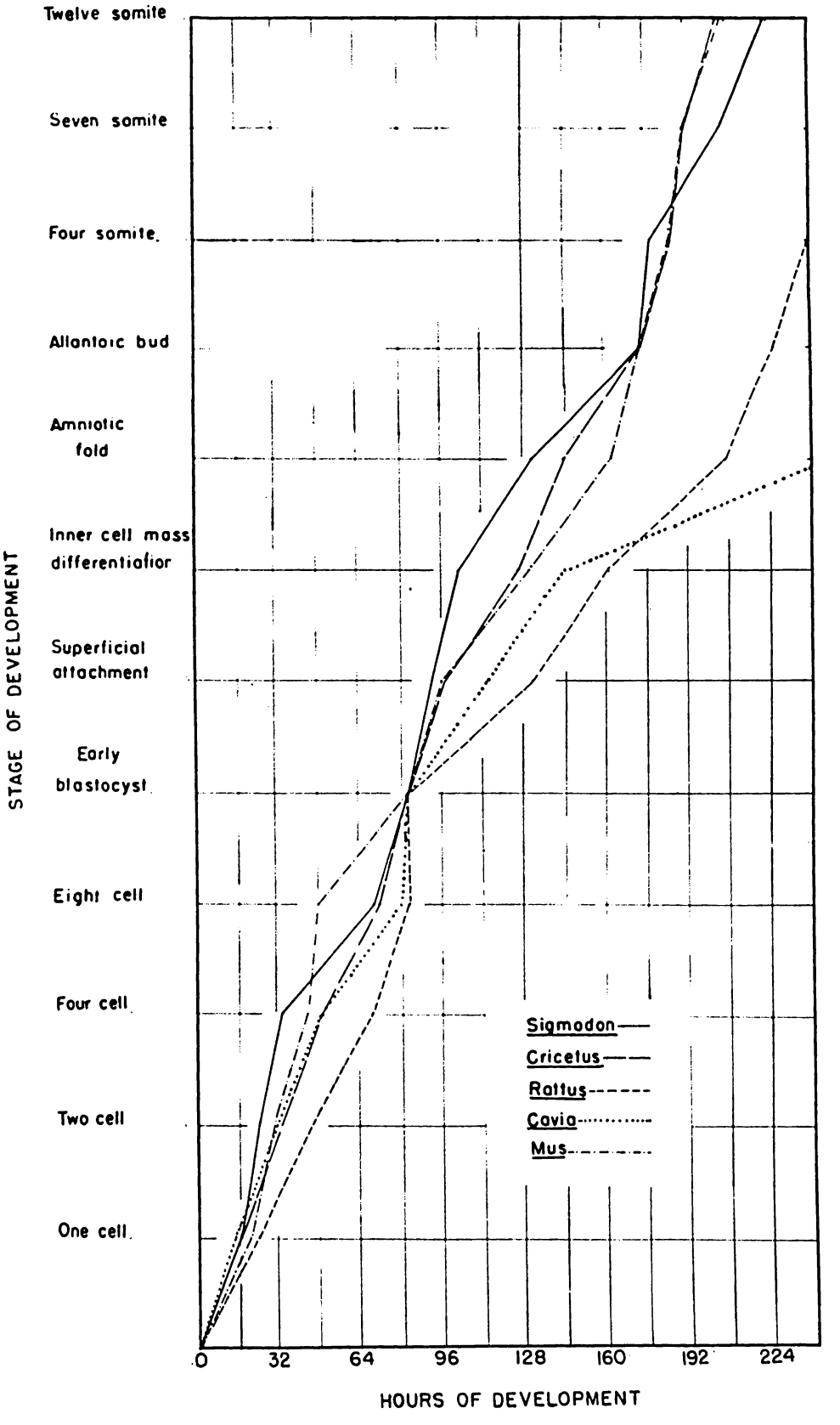
The trend of development in all the rodents has general similarities (figure 1: Huber, 1915; Snell, 1941; Long and Burlingame, 1938; Graves, 1945; Ward, 1948; Venable, 1946a; Harman and Prichett, 1932; Blandau, 1949a; Squier, 1932; McClaren and Bryce, 1926). Among the rodents compared there are variations in developmental rates. It is particularly significant that developmental rates in Cavia, Mus, Rattus, Cricetus, and Sigmodon almost coincide at 84 hours of development, the early blastocyst stage (figure 1). The cleaving egg, prior to implantation, seems to have approximately equal developmental potentialities in all the rodents compared.

In spite of the general similarities in developmental rates, there are some real differences. Through 38 hours of development Sigmodon has the most rapid rate of development of the rodents compared (figure 1). In the 38 to 84 hour interval Mus has the most rapid rate of development. By 84 hours Sigmodon reassumes the most rapid rate of development and this rate is not again exceeded by any other animal compared until 170 hours when Cricetus and Mus exceed Sigmodon in rate of development. The developmental rates after implantation in all rodents compared begin to diverge widely and correlate closely with the gestation period for each animal (figure 1).

The developmental rate of Sigmodon upon first inspection

is surprising, because this rodent has a gestation period as long as 27 to 28 days. Yet, this developmental rate is not surprising when one is cognizant of the state of development of the young at parturition and shortly thereafter. At parturition, Sigmodon has a furry coat; if alarmed, the young will run over the bottom of the cage a few hours after parturition; the eyes open in approximately two days after the young are born; the young feed on adult rations at eight days and they can be weaned by 15 days.

Figure I. Graph Showing Comparative Developmental Rates Among Some Rodents



PHYLETIC RELATIONSHIPS AMONG SOME RODENTS WITH SPECIAL CONSIDERATION OF *SIGMODON*

In order to use embryological data to show the relationships of *Sigmodon* to other rodents, Simpson's (1945) classification of the rodents is used because it seems to be the best system in the light of present knowledge. In his classification, *Sigmodon* would stand in relation to some other rodents as shown below.

Order Rodentia

Suborder Sciuromorpha

Superfamily Sciuroidea

Superfamily Geomyoidea

Family Geomyidae

Family Heteromyidae

Suborder Myomorpha

Superfamily Muroidea

Family Cricetidae

Subfamily Cricetinae

Tribe Hesperomyini *Sigmodon*

Tribe Cricetini *Cricetus*

Subfamily Microtinae *Microtus*

Family Muridae *Mus*, *Rattus*

Suborder Hystricomorpha

Superfamily Cavioidea

Family Caviidae *Cavia*

The significance of embryological data obtained from a study of Sigmodon, will be analyzed in the light of trends of evolution indicated by the embryology of rodents. These trends are reviewed below.

(1) In the Rodentia there is evolution from the giant-cell ring characteristic of primitive rodents to the "cone-shaped" ectoplacental cone characteristic of specialized rodents. The giant-cell ring is a constricted region where the bilaminar yolk sac joins the chorion and is observed in primitive Sciuroidea. In this evolutionary trend the giant-cell ring is modified into a syncytial ectoplacental cone, and evolutionary gradations of flat, "mushroom-shaped" or "cone-shaped" ectoplacental cones are observed. Flat ectoplacental cones are found in the Geomyoidea. "Mushroom-shaped" and "cone-shaped" ectoplacental cones are found in the Muroidea. "Cone-shaped" ectoplacental cones are observed in the Cavioidea.

(2) Gradations of inversion of the germ layers from a slightly inverted condition to that of complete inversion are observed. This trend is especially clear in the Sciuromorpha, where the splanchnopleuric yolk sac in the Sciuroidea is slightly inverted, while in the Geomyoidea the splanchnopleuric yolk sac is completely inverted and the definitive amniogenic region of the inner cell-mass is also inverted. Complete inversion of the inner cell-mass characterizes the Muroidea and the Cavioidea. Complete inversion results in inverted amnio-

genic and choriogenic regions as well as an inverted splanchnopleuric yolk sac.

(3) In the primitive Sciuroidea a small permanent allantoic vesicle occurs, which functions only to vascularize the chorion in contrast to the large endodermally lined, vesicular allantois in reptiles, which is used as a reservoir for waste materials as well as to vascularize the chorion. A nonvesicular allantoic cord characterizes the Geomyoidea, Muroidea and Cavioidea. As in the Sciuroidea, this allantoic cord serves only to vascularize the chorion, providing for exchange between the fetus and the mother.

(4) The placenta seems to have evolved from a hemochorial type to a hemo-endothelial type. In the hemo-chorial placenta of the Sciuroidea the endothelium of maternal blood vessels is destroyed and the fetal placental villi are situated in maternal blood labyrinths. In the hemo-endothelial placenta of the Geomyoidea, Muroidea, and Cavioidea the syncytial and connective tissues of the chorion have disappeared and fetal villi are separated from maternal blood only by the endothelium of chorionic blood vessels.

(5) Amniogenesis shows a remarkable evolutionary trend in the Rodentia. In the Sciuroidea there is a folding of the extraembryonic somatopleure in the formation of the chorion and amnion, as in reptilian embryos. In the Geomyoidea two stages of evolution of amniogenesis result from gradations of inversions: (a) Inversion of germ layers in the Geomyidae

involves only the amniogenic region of the inner cell-mass and the splanchnopleuric yolk sac; although small amniotic folds fuse, an open chorionic vesicle remains; (b) Inversion of the germ layers in the Heteromyidae involves not only the splanchnopleuric yolk sac and the amniogenic region of the inner cell-mass, but also most of the choriogenic region of the inner cell-mass. A result is the closing of the chorionic vesicle. The next trend in evolution of amniogenesis is a combination of modified folding and cavitation in the Muroidea where cavitation produces a chorio-amniotic cavity. Modified amniotic folds fuse to form the chorion and amnion which divide the chorio-amniotic cavity into chorionic and amniotic cavities and exocoelom. The final trend is cavitation alone in the Cavioidea where the chorionic and amniotic cavities are formed as distinct, separate cavities.

(6) The primitive type of implantation found in the Rodentia is eccentric and superficial, as in the Sciuroidea. In the Sciuridae the blastocyst attaches to the ventral side of uterine lumen in a uterine groove and then is closed off from the rest of the uterine lumen. In the Geomyoidea and Muroidea, implantation is eccentric at first and finally partly interstitial, an intermediate situation. In these groups of animals the blastocyst usually comes to be situated in a ventral uterine groove, the blastocyst becomes closed off from the remainder of the uterine cavity and erodes into the

endometrial tissue of this region. Interstitial implantation indicates specialization and is characteristic of the Cavioidea. In the Cavioidea the blastocyst erodes into a sub-epithelial location in the decidua without first being closed off in a uterine groove.

(7) The bilaminar yolk sac, which is the endodermally-lined trophoblast of young blastocysts, persists until full-term in the Sciuroidea. The bilaminar yolk sac disappears at approximately mid-term in the Geomyoidea; in the Muroidea the bilaminar yolk sac disappears in the early fetal stages. The bilaminar yolk sac does not exist in the Cavioidea.

Of the evolutionary trends discussed, these characterize Sigmodon: (a) Complete inversion of the inner cell-mass and the yolk sac; (b) A spongy allantoic cord; (c) A hemo-endothelial placenta; (d) An ephemeral yolk sac probably disappearing in early fetal stages; (e) Amniogenesis by cavitation and folding; (f) Early eccentric implantation followed by partly interstitial implantation and (g) A "cone-shaped" ectoplacental cone. The expression and probable significance in the ontogeny of these characteristics of Sigmodon are discussed briefly below.

(a) In the early development of Sigmodon the inner cell-mass grows deep into the blastocoel with the result that proximal endoderm almost fuses with distal endoderm. Furthermore inversion in Sigmodon embryos places the tissues which give rise to the amnion, chorion, yolk sac and ectoplacental

cone, in the inner cell-mass. Complete inversion is also observed in the early development of Cricetus, Mus, Rattus and Microtus. Complete inversion occurs in Cavia, but in this genus the amniogenic region is distinct from the rest of the inner cell-mass at an earlier relative time than in the development of the genera mentioned above. Complete inversion occurring in the early ontogeny indicates that Sigmodon is relatively specialized in this respect, as compared with Dipodomys and Geomys where only partial inversion occurs in early development. Sigmodon seems to be highly specialized in comparison with Citellus and Sciurus in which inversion involves only a part of the splanchnopleuric yolk sac. Complete inversion in early ontogeny is a feature common to Sigmodon, Cricetus, Mus, Rattus, and Microtus which seems to confirm the current superfamilial classification of these genera.

(b) The allantoic cord is spongy in structure, but never vesicular in the development of Sigmodon. This is also true in the early development of Cricetus, Mus, Rattus, Microtus, Cavia, Geomys and Dipodomys. It is only in the development of such rodents as Citellus and Sciurus that the allantois is vesicular. The structure of the allantois of Sigmodon is of little value in the classification of this genus, because a non-vesicular, cord-like allantois occurs in the early development of genera distributed among the three suborders of Rodentia. In the suborder Sciuromorpha only the superfamily Geomyoidea has the spongy allantoic cord in early

development. However, Sigmodon seems to be relatively specialized in this respect as compared with Citellus and Sciurus in which the allantois is vesicular.

(c) By the tenth day of development the placenta of Sigmodon is approaching a hemo-endothelial condition. The hemo-endothelial placenta of Sigmodon is similar to that of Mus, Cricetus, Rattus, Cavia, Geomys and Dipodomys. Only such rodents as Citellus and Sciurus differ from Sigmodon and they have hemo-chorial placentae. The hemo-endothelial placenta is considered to be an indication of specialization since it provides for a maximum facility of exchange between the embryo and maternal tissues with a relatively small placenta.

(d) It has previously been stated that this study does not cover a large enough interval of the development of Sigmodon to be sure of the fate of the bilaminar yolk sac, but the bilaminar yolk sac probably disappears in early fetal stages. It disappears in early fetal life in Mus, Rattus, Cricetus, and Microtus. Cavia differs from Sigmodon, however, in that the distal endoderm never appears and there is no bilaminar yolk sac. In Citellus and Sciurus the bilaminar yolk sac is retained full-term. The probable disappearance of the bilaminar yolk sac in Sigmodon in early fetal stages and the disappearance of the bilaminar yolk sac in Mus, Rattus, Cricetus and Microtus may indicate a relatively close relationship of Sigmodon with these genera. This phenomenon seems to

be an indication of specialization because it allows the embryos to have a more intimate contact with maternal tissues than would be possible if the bilaminar yolk sac remained until completion of the period of gestation.

(e) In the early ontogeny of Sigmodon, an amniotic cavity appears by cavitation in the embryonic ectoderm of the inner cell-mass, after which a chorionic cavity appears by cavitation in the extraembryonic ectoderm of the inner cell-mass. The amniotic and chorionic cavities immediately coalesce, and this cavity is finally divided into the chorionic and amniotic cavities and the exocoelom by modified amniotic folds. A similar phenomenon occurs in the early development of Mus, Rattus, Microtus and Cricetus. The early development of Cavia differs from the early development in Sigmodon in that the chorionic and amniotic cavities remain separate, and folding cannot occur. Amniogenesis in Geomys, Dipodomys, Sciurus and Citellus differs from this phenomenon in Sigmodon in that it occurs by folding of the extraembryonic somatopleure. Amniogenesis by cavitation and folding seems to indicate a relatively close relationship among Sigmodon, Mus, Rattus, Microtus and Cricetus and seems to confirm the current superfamilial classification of Sigmodon as well as relatively closely related genera. Furthermore, amniogenesis by cavitation and folding indicates relative specialization of the Muroidea as compared with the Geomyoidea and the Sciuroidea. However, amniogenesis by cavitation alone indicates that the Cavioidea are relatively the most specialized of rodents in

this respect.

(f) The process of implantation in Sigmodon is at first eccentric, followed by partly interstitial implantation. Blastocysts of Sigmodon are found in ventral uterine grooves or at least in a ventral part of the uterine lumen. Here the blastocysts are closed off from the uterine lumen as the decidual reaction produces a swollen implantation site. After the blastocysts are closed off from the uterine lumen, erosion of the endometrium by the blastocyst occurs. A similar phenomenon occurs in Mus, Rattus, Cricetus, Microtus, Geomys and Dipodomys. The phenomenon of interstitial implantation provides for close contact with maternal tissue by the fetal tissues. Cavia, on the basis of this criterion, is relatively the most specialized of rodents, for the embryo erodes into uterine tissue without being closed off in a uterine groove. In this respect Sigmodon and genera which have a similar type of implantation are less specialized.

(g) The nature of the ectoplacental cone is of particular significance with reference to the classification of Sigmodon. Sigmodon, as well as Mus, Rattus, and Microtus, has a "cone-shaped" ectoplacental cone which consists of syncytial tissue and relatively few giant cells and these giant cells are small as compared with primary giant cells. Cricetus, a member of the same subfamily as Sigmodon, has a "mushroom-shaped" ectoplacental cone consisting of cords of vacuolated cells which gives rise to relatively small giant cells. Differences in the ectoplacental cone in the early

development of Sigmodon and Cricetus are the only basis for differentiating between the two genera. With respect to the nature of the ectoplacental cone Cricetus seems to be more primitive than Sigmodon.

Generalizations concerning the hypothetical relationships of some rodents, based on the trends of evolution as indicated by development, are stated below and the hypothetical relationships are diagrammed in figure 2.

(1) Ontogeny in Rodentia displays variations and seeming evolutionary trends which strongly indicate that the higher categories of the current classification (Simpson, 1945) are natural groupings.

(2) Because its ontogeny is characterized by amniogenesis by folding of the extraembryonic somatopleure, a giant-cell ring, eccentric and superficial implantation, a hemo-chorial placenta, a bilaminar yolk sac retained until full-term and an allantoic vesicle, I judge that the superfamily Sciuroidea is one of the most primitive groups of rodents.

(3) Because of amniogenesis by degrees of folding, complete inversion of the splanchnopleure yolk sac and gradations of inversion of the inner cell-mass, a flat ectoplacental cone, and a bilaminar yolk sac until mid-term, it seems that the Geomyoidea occupy an intermediate evolutionary position and may possibly have been the source of rodents now classified in the suborders Myomorpha and Hystricomorpha.

(4) Because of complete and extreme inversion of the amniogenic region of the inner cell-mass, interstitial implantation, an absence of a bilaminar yolk sac, and amniogenesis by cavitation alone, the Cavioidea seem to be the most specialized group of rodents.

(5) Because Sigmodon, Cricetus, Microtus, Mus and Rattus are characterized by partly interstitial implantation, a bilaminar yolk sac until early fetal stages and amniogenesis by cavitation and folding, embryological criteria confirm the current superfamilial classification of these genera.

(6) Sigmodon, Microtus, Mus and Rattus may be more closely related than current classification indicates, since they are similar in all observed developmental characteristics.

(7) The relative specialization of Sigmodon as compared with Cricetus is indicated by the appearance of a "cone-shaped" ectoplacental cone in early ontogeny of the former and a "mushroom-shaped" ectoplacental cone in early development of the latter.

The use of embryological data in phylogenetic studies of the Rodentia is limited because the development of relatively few rodents has been investigated, the evolution of the morphology of embryonic characteristics is conservative, and differences between groups of rodents are small, particularly on the subfamilial level or lower, and because it is difficult to detect convergent or parallel evolution.

In the suborder Hystricomorpha, the development of Cavia alone has been investigated. In the suborder Myomorpha, the development of Cricetus, Mus, Rattus, Microtus and Sigmodon has been investigated. The ontogeny of Peromyscus, Fiber, Neotoma and Reithrodontomys has been so slightly investigated that the information from these genera is of only limited value. Among the Sciuromorpha, the development of Sciurus, Citellus, Geomys and Dipodomys has been investigated. The ontogeny of a few other rodents such as Castor, and Tamias has been investigated less thoroughly. Most of the investigations concerned with ontogeny in genera of the Sciuromorpha have been done with uteri collected and preserved in the field and these investigations have been concerned mostly with fetal membranes. The use of well-preserved uteri taken from rodents which have been trapped in the field is adequate for understanding the ontogeny and morphology of the fetal membranes where age of embryos is not espedially important. This eliminates the time-consuming work of learning how to raise various native rodents in the laboratory. Embryologists and collectors of mammals could well cooperate more frequently in order to add to a knowledge of the comparative embryology of rodents.

SUMMARY

1. Ontogeny in Rodentia displays variations and

evolutionary trends which strongly indicate that the higher categories of the current classification (Simpson, 1945) are natural groupings.

2. The superfamily Sciuroidea of the suborder Sciuromorpha contains the most primitive rodents.

3. The superfamily Geomyoidea of the suborder Sciuromorpha occupies an intermediate evolutionary position and might possibly have been the source of the rodents which are classified in the suborders Myomorpha and Hystricomorpha.

4. The Cavioidea of the suborder Hystricomorpha are among the most specialized rodents.

5. Embryological criteria confirm the current superfamilial classification of Sigmodon, Cricetus, Microtus, Mus and Rattus.

6. Sigmodon, Microtus, Mus and Rattus may be more closely related than current classification indicates.

7. Sigmodon is relatively specialized as compared with Cricetus.

Figure 2. A Diagram Showing Hypothetical Relationships Among Some Rodents Based on Embryological Data.

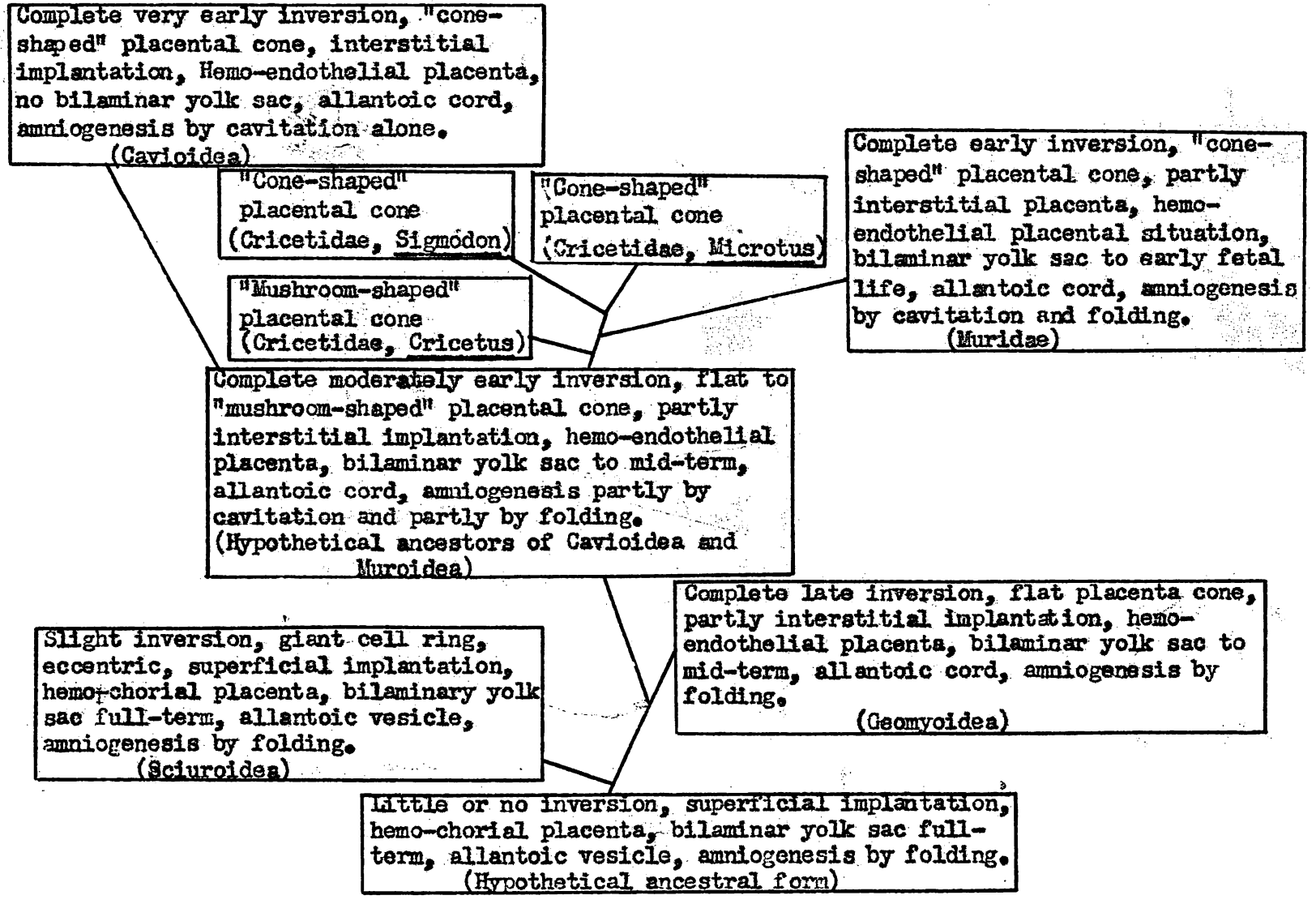


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Plate I

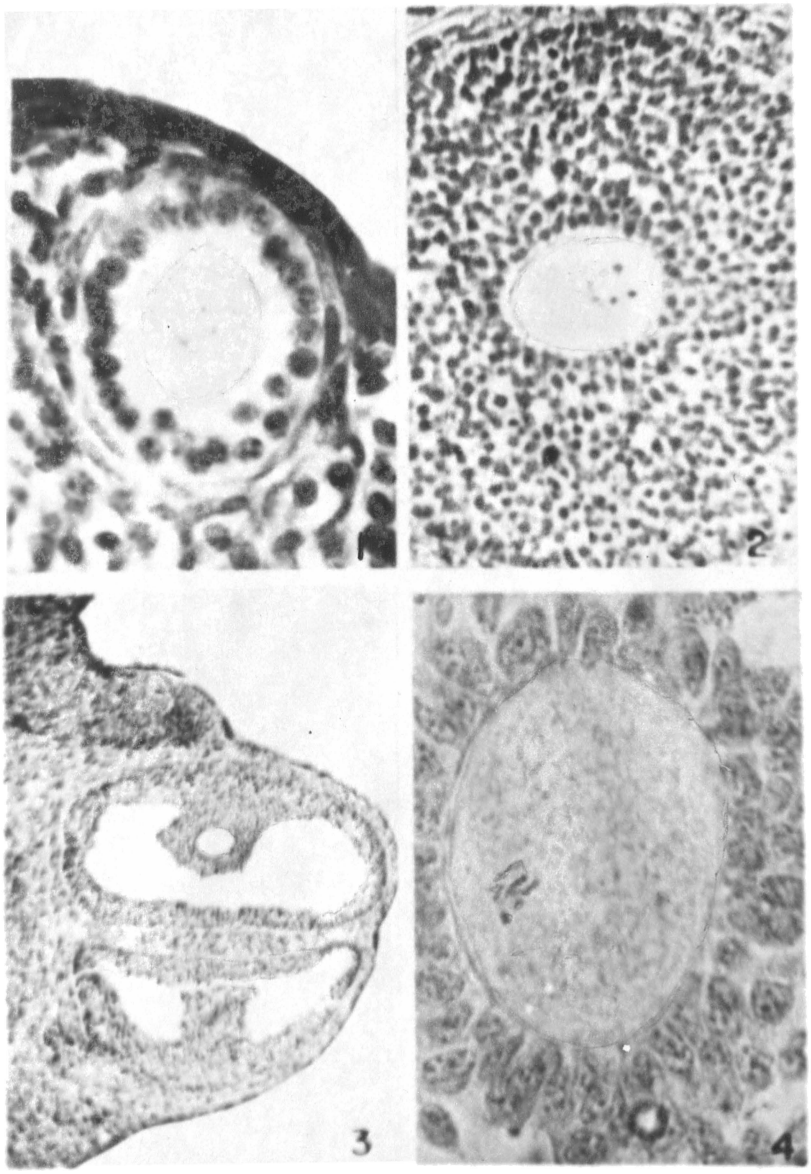

Figure 1. Primary follicle. X534.

Figure 2. Primary follicle with spaces appearing among the follicle cells. X240.

Figure 3. Mature secondary follicle. X53.

Figure 4. Primary oöcyst in prophase of the first meiotic division. X560

Plate II

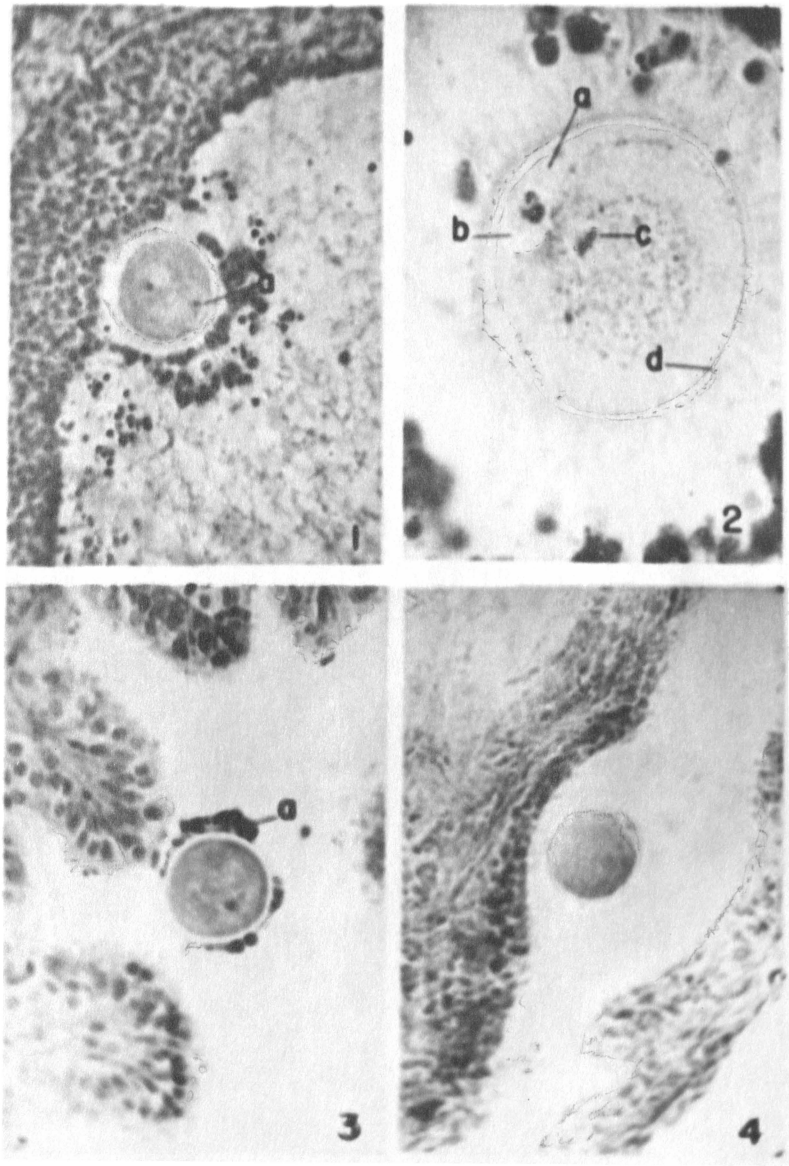

Figure 1. Primary oöcyst im metaphase of the first meiotic division. a, Spindle. X228.

Figure 2. Floating ovum in the mature ovarian follicle. a, Perivitelline space; b, First polar body; c, Metaphase spindle of the second meiotic division; d, Zona pellucida. X515.

Figure 3. Newly ovulated ovum with part of the corona radiata still adhering. a, Corona radiata. X200.

Figure 4. Tubal ovum after copulation has taken place at 10-15 hours. X150.

Plate III

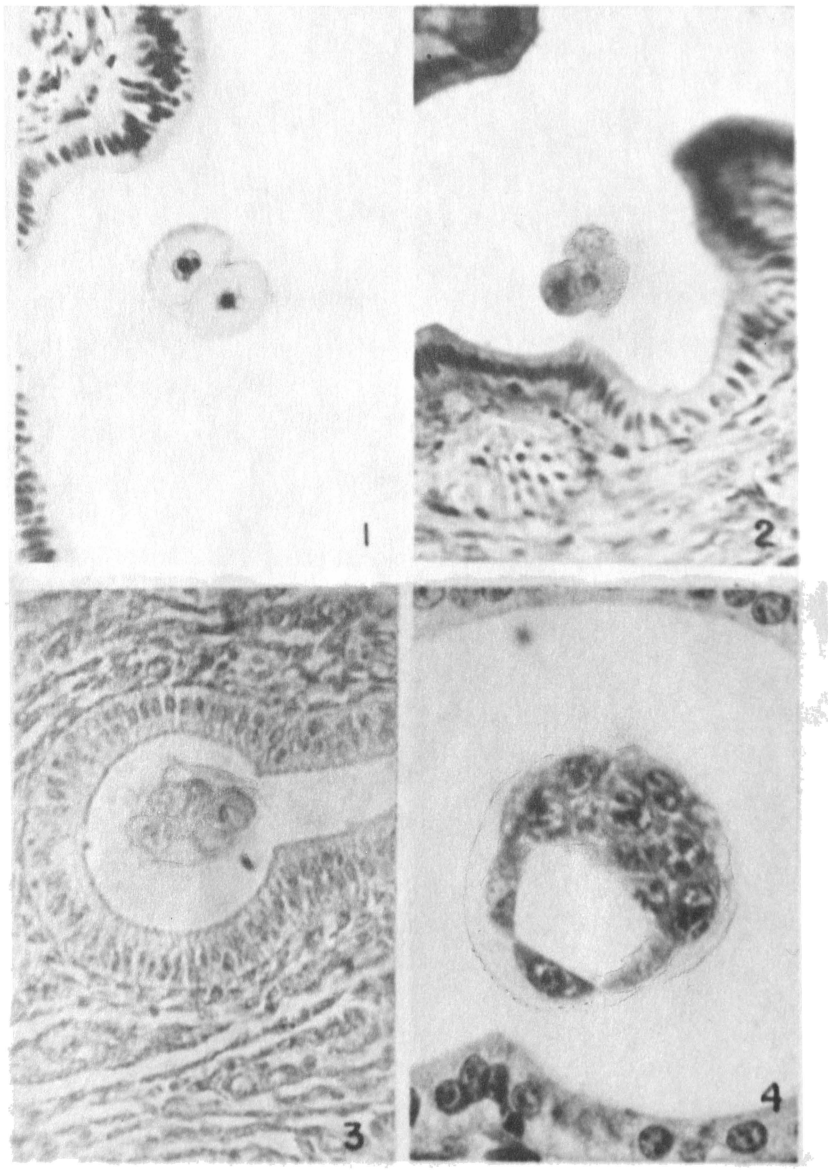

Figure 1. Two-cell stage at 24 hours. X150.

Figure 2. Three to four-cell stage at 36 hours. X150.

Figure 3. Eight-cell stage. 72 hours. X180.

Figure 4. Early blastocyst. Three and one half days. X457.

Plate IV

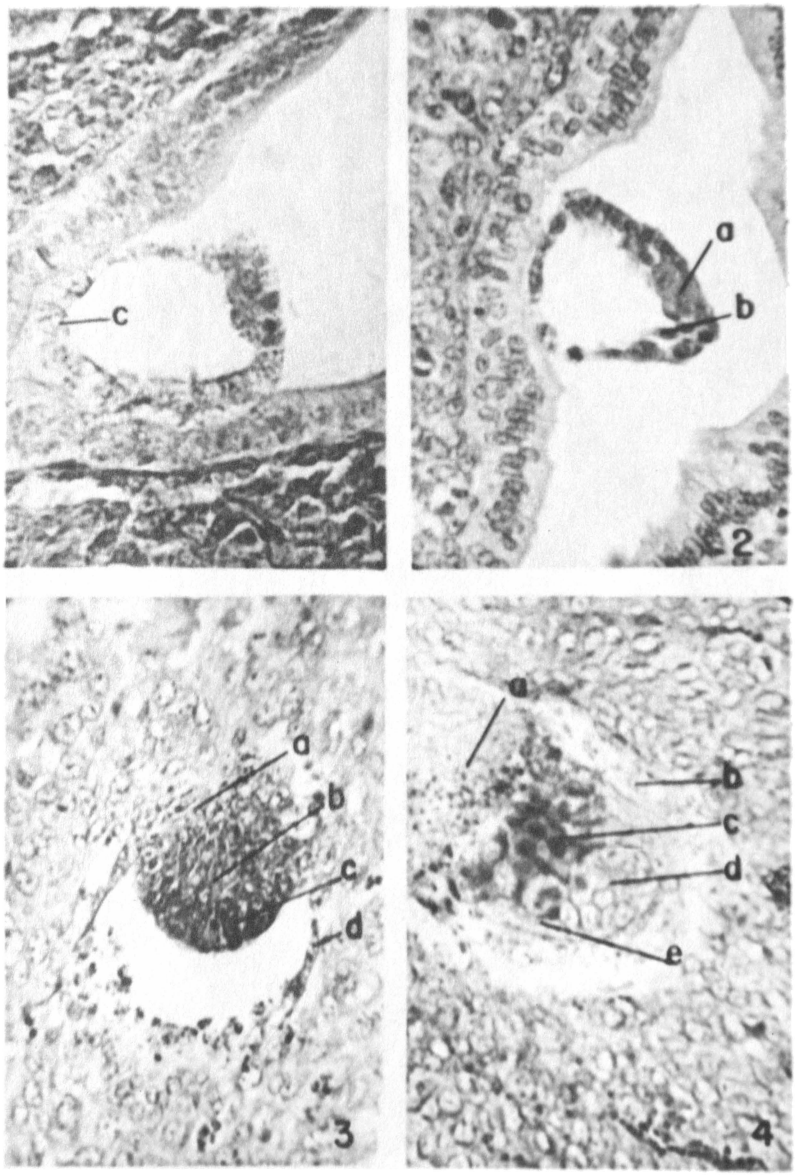

Figure 1. Blastocyst in early implantation at three days 16 hours. c, Enlarged trophoblast cell. X200.

Figure 2. Blastocyst in early implantation and endoderm formation at three days 16 hours. a, Inner cell mass; b, Endoderm. X200.

Figure 3. Penetration of uterine epithelium and inner cell mass differentiation at four days. a, Extraembryonic ectoderm; b, Embryonic ectodermal node; c, Endoderm; d, Trophoblast. X160.

Figure 4. Furthur differentiation of the inner cell mass and ectoplacental cone formation at four days four hours. a, Ectoplacental cone; b, Trophoblast cells; c, Extraembryonic ectoderm; d, Embryonic ectoderm; e, Endoderm. X200.

Plate V

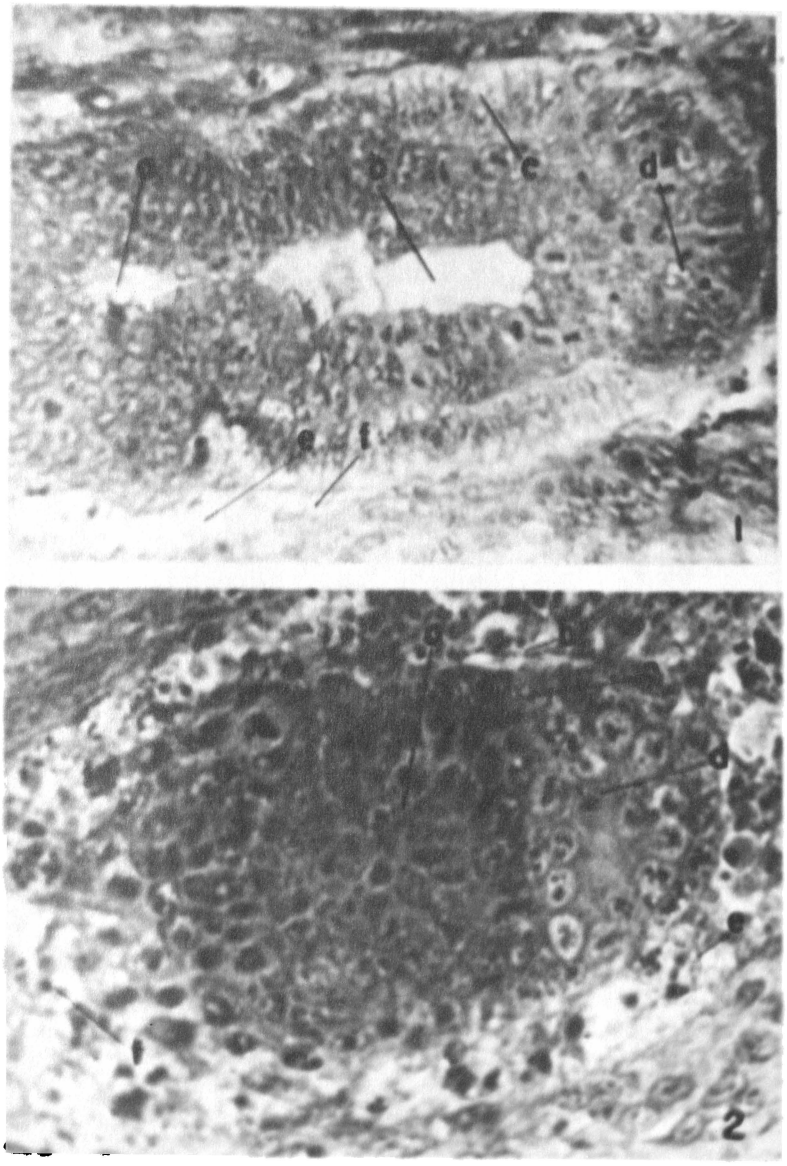

Figure 1. Longitudinal section through a five-day embryonic cyst. a, Ectoplacental cavity; b, Amnio-chorionic cavity; c, Proximal endoderm; d, Embryonic ectoderm; e, Old blastocoel; f, Reichert's membrane. X315.

Figure 2. Longitudinal section through a four and one half day embryo. a, Extraembryonic ectoderm; b, Distal endoderm; c, Proximal endoderm; d, Embryonic ectoderm; e, Primary giant cell; f, Ectoplacental cone. X315.

Plate VI

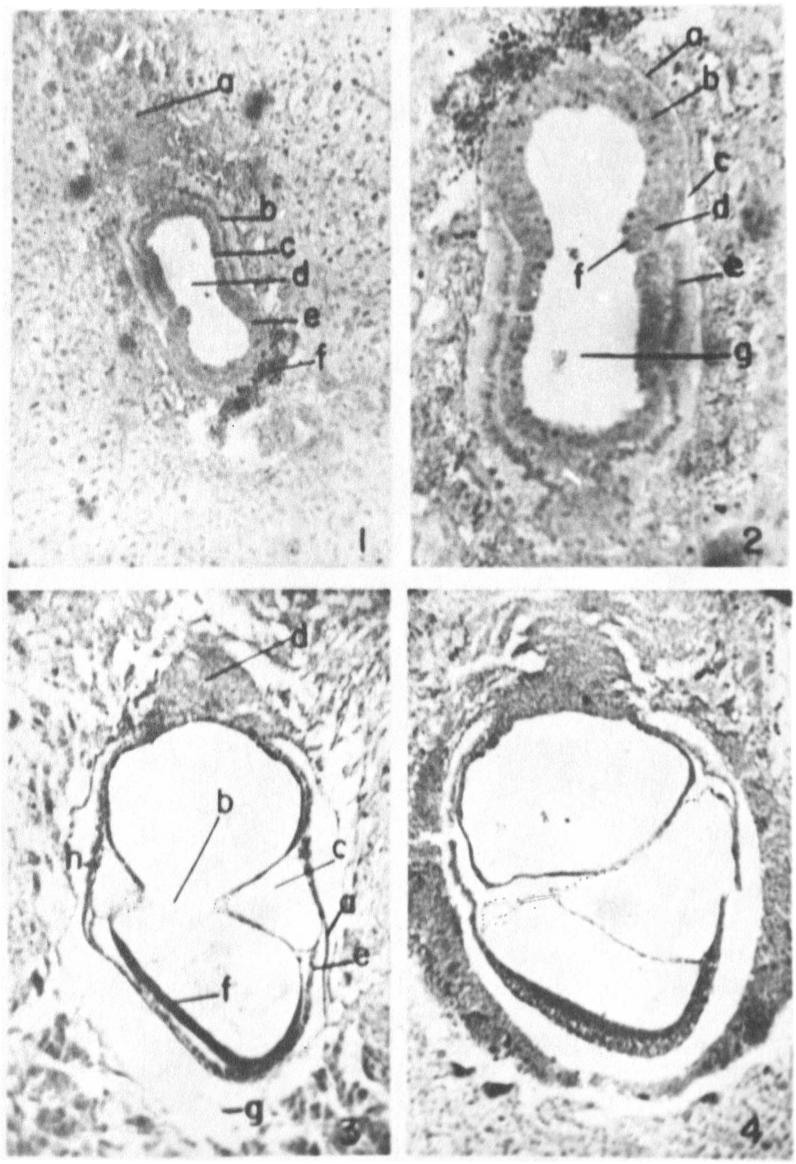

Figure 1. Median sagittal section of embryonic cyst at six days. a, Ectoplacental cone; b, Proximal endoderm; c, Extraembryonic ectoderm; d, Amnio-chorionic cavity; e, Embryonic ectoderm; f, Blood sinus. X75.

Figure 2. Same as figure 1, but greater magnification. a, Reichert's membrane; b, Embryonic ectoderm; c, Old blastocoel; d, Mesoderm; e, Proximal endoderm; f, Amniotic fold; g, Amnio-chorionic cavity. X200.

Figure 3. Median sagittal section of embryonic cyst at seven days. a, Proximal endoderm; b, Amnio-chorionic pore; c, Exocoelom; d, Ectoplacental cone; e, Mesoderm; f, Embryonic ectoderm; g, Distal endoderm and Reichert's membrane; h, Yolk sac. X54.

Figure 4. Longitudinal section of embryonic cyst at seven days. X54.

Plate VII

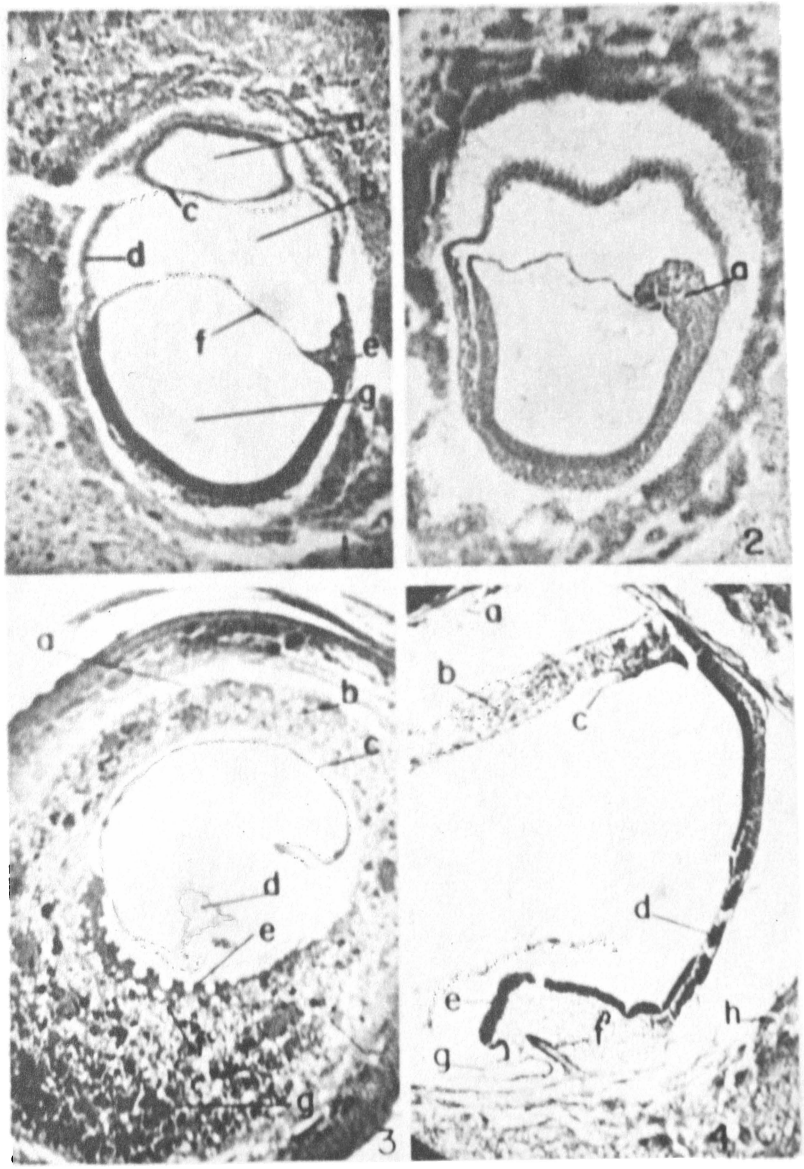

Figure 1. Longitudinal section of the embryonic cyst at seven days. a, Chorionic cavity; b, Exocoelom; c, Chorion; d, Yolk sac; e, Allantoic bud; f, Amnion; g, Amniotic cavity. X54.

Figure 2. Oblique section along the longitudinal axis at seven days. a, Allantois. X54.

Figure 3. Placental site of a eight and one half day embryo. a, Uterine lumen reappearing; b, Decidua capsularis; c, Yolk sac; d, Allantois; e, Trophoblastic tubules; f, Maternal sinusoids g, Decidua basalis. X18.

Figure 4. Longitudinal section of a four-somite embryo at seven and one half days. a, Yolk sac; b, Allantois; c, Amnion; d, Somite; e, Head fold; f, Foregut; g, Endothelial tubes of the heart. X68

Plate VIII

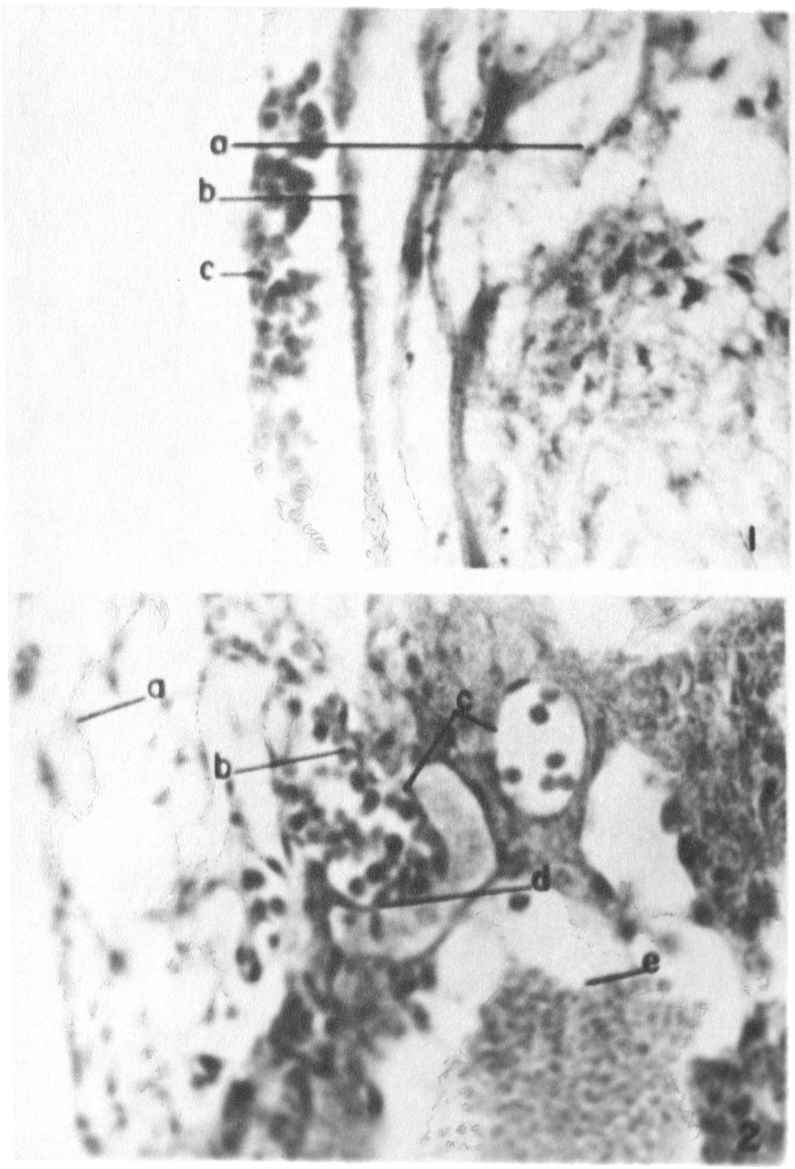

Figure 1. Early stage of angiogenesis at seven and one half days. a, Uterine tissue; b, Yolk sac endoderm; c, Yolk sac mesoderm in which angiogenesis is beginning. X185.

Figure 2. Placental circulation and hemopoesis at nine and one half days. a, Allantoic tissue; b, Hemoblasts; c, Trophoblastic vessels; d, Fetal blood vessel lining approaching a hemo-endothelial condition; e, Maternal sinusoid containing erythrocytes. X185.

Plate IX

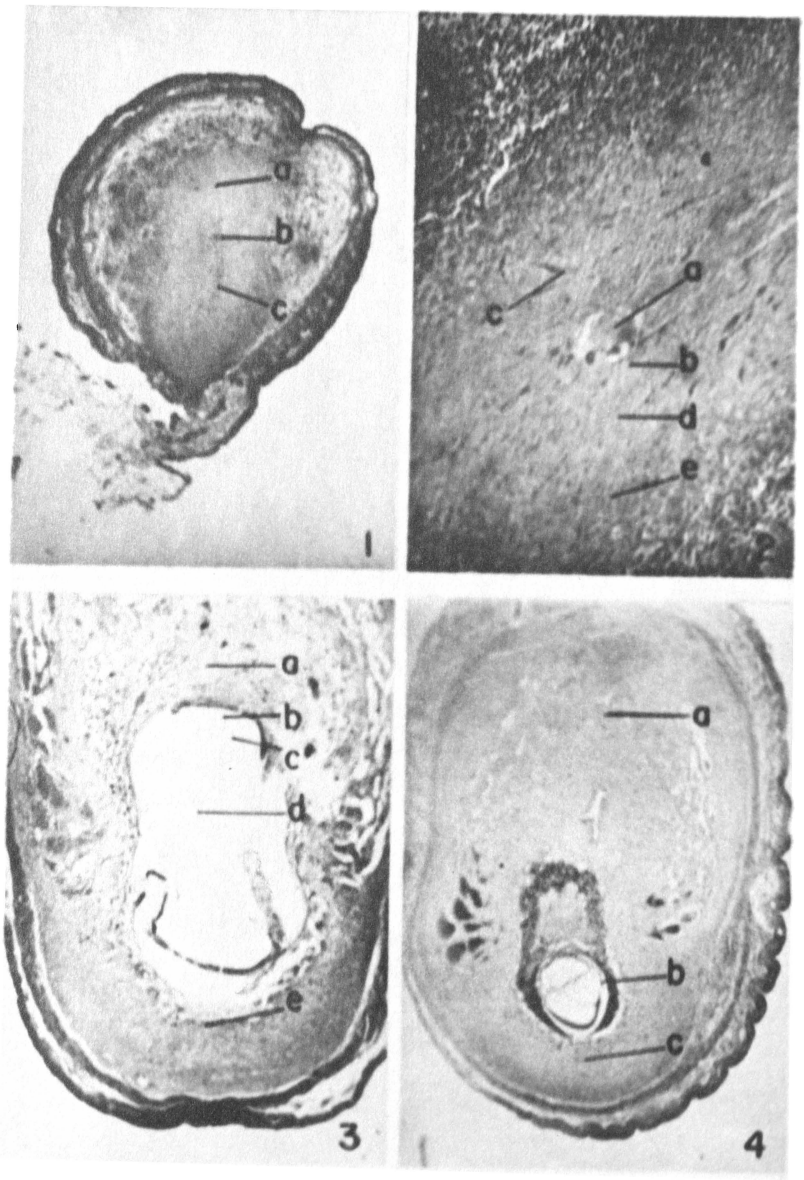

Figure 1. Transverse section of the uterus at the implantation site. Four days four hours. a, Decidua capsularis; b, Embryo; c, Decidua basalis. X21.

Figure 2. Enlarged section of implantation region at four days four hours. a, Embryo; b, Inner decidual zone; d, Intermediate decidual zone; e, Outer decidual one; c, Radiating sinuses. X53.

Figure 3. Transverse section of the implantation site at seven and one half days. a, Placental region; b, Chorionic cavity; c, Chorion; d, Amnion; e, Decidua capsularis. X25.

Figure 4. Transverse section of the implantation site at seven days. a, Decidual basalis; b, Embryo; c, Decidua capsularis. X17.

Plate X

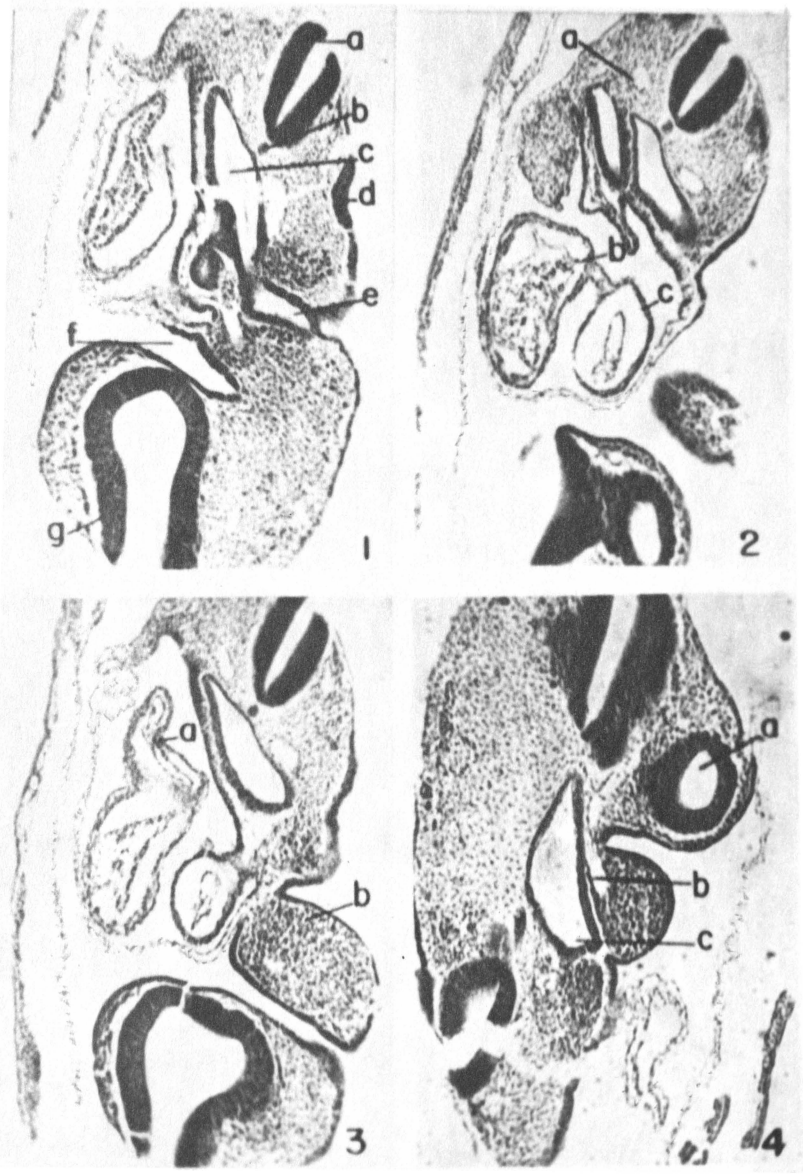

Figure 1. Transverse section of an eight and one half day embryo. a, Open myelencephalon; b, Notochord; c, Pharynx; d, Otic placode; e, First pharyngeal pouch and closing membrane; f, Stomodaeum; g, Telencephalon. X77.

Figure 2. Transverse section of an eight and one half day embryo. a, Paired dorsal aortae; b, Ventricle with blood cells in it; c, Bulbus. X77.

Figure 3. Transverse section of an eight and one half day embryo. a, Atrium; b, Mandibular arch. X77.

Figure 4. Transverse section of an eight and one half day embryo. a, Optic vesicle; b, Aortic arch; c, Pharynx leading into a pharyngeal pouch. X77.

Plate XI

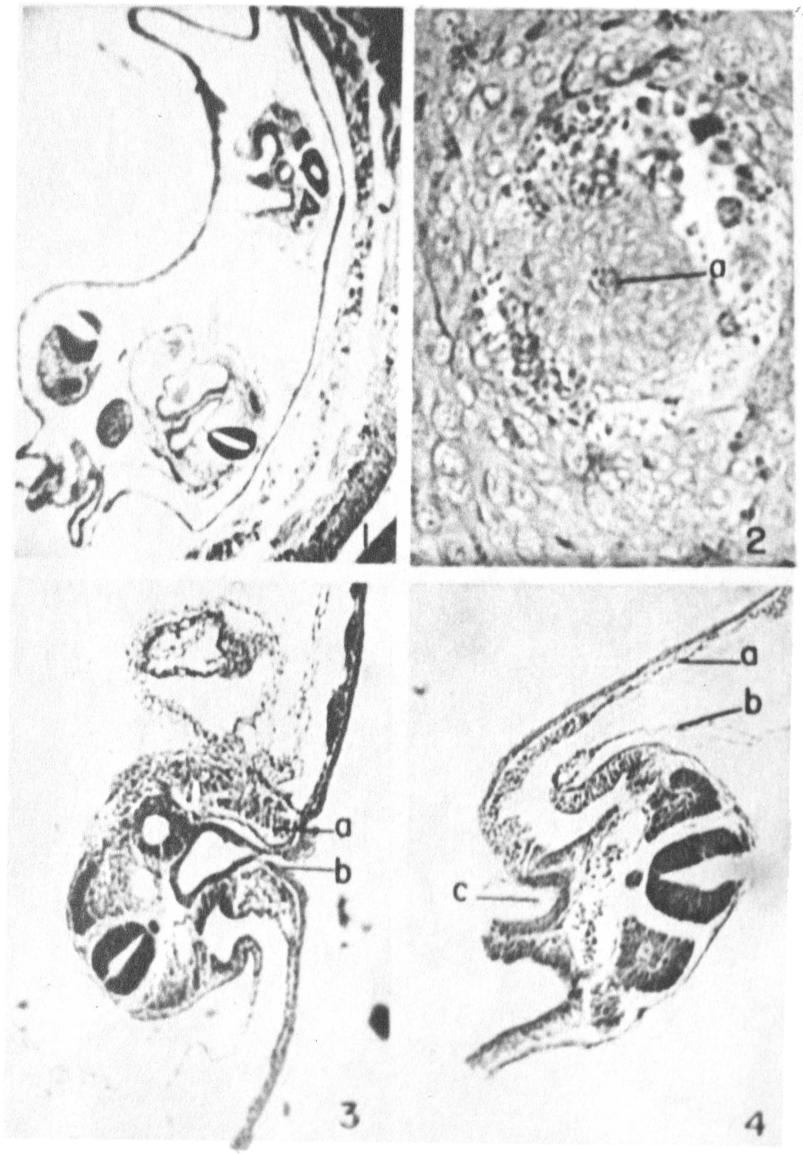

Figure 1. Section through the cephalic and caudal regions of an eight and one half day embryo showing flexure and torsion. X26.

Figure 2. Transverse section through a four day four hour embryo. a, Proamniotic cavity. X75.

Figure 3. Transverse section through aneight and one half day embryo. a, Vitelline veins; b, Anterior intestinal portal. X77.

Figure 4. Transverse section through aneight and one half day embryo. a, Yolk sac; b, Amnion; c, Mid-gut. X77.