THE DEVELOPMENT, HISTOLOGY AND CIRCULATORY PATTERN OF THE CHINCHILLA PLACENTA

by

FORREST DONALD TIBBITTS

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APPROVED:

Redacted for privacy

Professor of Zoology

In Charge of Major Redacted for privacy

Chairman of Zoology Department

Redacted for privacy Chairman of School Graduate Committee

Redacted for privacy

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THE DEVELOPMENT, HISTOLOGY AND CIRCULATORY PATTERN OF THE CHINCHILLA PLACENTA

INTRODUCTION

The domestic chinchilla (<u>Chinchilla lanigera</u>, Bennett) belongs to the rodent family Chinchillidae, which, together with thirteen other rodent families, forms the Suborder Hystricomorpha. Of these fourteen families only the guinea pig is well known with respect to the development of the embryonic adnexa, largely through the investigations of Duval (11, p. 477-548), Sansom and Hill (24, p. 295-351), and Amoroso (3, vol. 2, p. 270-276). With the exception of the porcupine (<u>Erethizon dorsatum</u>, Fam. Erethizontidae) which has recently been studied by Perrotta (21, p. 345-346), and the work of Strahl (26, p. 524-528) and Becher (6, p. 337-364; 7, p. 439-454) on the agouti (<u>Dasyprocta azarae</u>, Fam. Dasyproctidae) the placental anatomy and development is unknown for the remaining hystricomorph rodents.

Similarly, the patterns of the maternal and fetal blood streams in the complex lobate placenta of the Hystricomorpha have received little attention, although these vascular arrangements have been the subject of detailed investigations in the placentae of the rat (9, p. 356-368; 10, p. 369-375), hamster (2, p. 52-53), ground squirrel (18, p. 165), and rabbit (17, p. 433-497). Becher makes reference to the major maternal and fetal blood vessels in the agouti placenta, as does Duval in discussing placentation in the guinea pig. However, neither of these authors dwell at length on the over-all fetal-maternal circulatory patterns in the placenta. Hard (15, p. 77) presents a diagram of blood vessel relationships in the placental exchange area of the guinea pig, but this sketch is primarily to show the distribution of the enzyme, alkaline phosphatase.

MATERIALS AND METHODS

Forty-four female chinchillas in various stages of pregnancy were prepared for study by the methods outlined below.

A total of nine placentae were injected intravascularly with various color combinations of latex and vinyl resin and either corroded with KOH or cleared in KOHglycerine to reveal the vascular relationships within the placentae. The vinyl casts were preferred to the latex preparations since the rigidity of the former provided a more faithful spatial relationship among the blood vessels.

Another series of mature placentae were injected with variously colored gelatin masses and India ink. These were embedded in paraffin and serially sectioned at 10-15 microns. These sections when lightly stained with hematoxylin and eosin made it possible to observe the finer vascular structure and associations within the substance of the placentae.

A number of small loculi were immersed in Bouin's fluid and among these a few were also injected intravascularly through the maternal and/or fetal vessels. Blocks through the central portions of the placentae were cut, embedded in paraffin, sectioned at 10 microns, and stained with either hematoxylin and eosin, Masson's trichrome

stain, or iron hematoxylin and light green.

It is well to comment briefly on the problems associated with obtaining accurately dated stages of pregnancy in the chinchilla in explanation for the gaps in the present series of placentae.

To breed females successfully it is essential that they be paired with males sometime <u>prior</u> to the period of estrus, since a female usually will not tolerate a newly introduced male. In the colony of chinchillas maintained at Oregon State College it was impractical to pair the animals, primarily because of an insufficient number of proven males and the risk of "wasting" the few good males by placing them with non-cycling females.

As an alternative to natural matings a series of artificial inseminations was attempted, employing a procedure developed by Dr. Howard H. Hillemann of Oregon State College. Although the technical details of this method are very satisfactory there is as yet no accurate method for determining the time of ovulation in this animal; this situation greatly reduces the chances of pregnancy resulting from artificial insemination. However, out of twelve females artificially inseminated post partum one did become pregnant.

In the few cases of successful natural matings in the colony the gestation age of the placentae was determined

from the time of observation of the copulatory plug or other evidence of mating. All other placentae were obtained from females found to be pregnant upon arrival from breeders. Because it is not the practice of the commercial raisers to keep records of matings the precise gestation ages of all but a few of these placentae are unknown. These placentae were placed in relative chronological sequence on the basis of crown-rump measurement, weight, and various developmental features.

I. THE DEVELOPMENT OF THE VITELLINE AND ALLANTOIC PLACENTAE

The Non-Pregnant Uterus

Two previous studies by Beyerlein and Hillemann (8, p. 8-10) and Roos and Shackelford (23, p. 301-312) have dealt in detail with the gross reproductive anatomy of the female chinchilla.

The uterus of the chinchilla is duplex, the horns of which open by two lateral slit-like ora cervicis into the vagina. The broad ligaments suspend the uterine horns from the dorsal coelomic wall and carry the blood vessels which serve the uterine horns and later also the fetus via the maternal placenta. The thin outer longitudinal muscle layer of the uterus may extend for a short distance into the mesometrium; this extension is particularly marked in parous females, in comparison with the virgin, and may have a functional significance in the gravid female in terms of providing for uterine distension.

The inner circular muscle layer is relatively thicker than the outer longitudinal layer and is separated from the latter by a zone of loose connective tissue through which pass the major circumferential blood vessels of the uterine wall.

When viewed in cross section the uterine mucosa is

cast into a series of low folds which in reality are transverse crescentic plicae. These plicae tend to divide the uterine horns into irregular segments. In fresh uteri <u>in situ</u> these folds are not readily apparent, but after removal from the animal, and especially after fixation, the contraction of the uterine smooth muscle enhances their outline. These crescentic folds never completely disappear during pregnancy and regularly enlarge at both the tubal and cervical poles of the loculus where they may be functional in preventing excess movement of the placenta; this function has been previously ascribed to similar folds in the uterus of the agouti by Becher (6, p. 341-342).

The mucosa is covered by a simple columnar epithelium continuous with the slender-necked simple tubular glands which open into the uterine lumen.

Stage 1. Egg Cylinder, Thirteen Days Gestation

The youngest stage available in this study is that represented by two loculi of thirteen days gestation (vaginal plug determination). The tubular egg cylinder occupies the upper half of the hour-glass shaped implantation chamber and is so oriented that it lies in the plane of the mesometrium with the ectoplacental trophoblast mesometrial and the amnioembryonic mass antimesometrial.

Both the epamniotic cavity (chorionic cyst, ectoplacental cavity, false amniotic cavity) and the amniotic cavity are formed independently by cavitation, as in the guinea pig (11, p. 242), rather than by folding as in the rat (11, p. 302) or hamster (1, p. 365). At thirteen days the epamniotic cavity is well developed and frequently filled with extravasated maternal blood. although the amniotic cavity is not yet evident. At the mesometrial pole of the egg cylinder the layer of chorionic ectoderm ("lamina" of Everett) (12, p. 252) is continuous with the ectoplacental trophoblast (outer) by a loose association of trophoblast cells extending through the central region of the epamniotic cavity (Plate 1, figs. 1, 2, 3 and 4; Plate 14, fig. 3). It may be that the epamniotic cavity originated by the coalescence of several small peripheral cavities leaving an intact central loose cellular region.

However, it may also be argued that this loose bridge of cells is developed secondarily across the cavity by apposition of the outer and inner trophoblast layers as in the guinea pig (11, fig. 214). Since earlier stages of development are not now available no definite choice can be made between the alternative interpretations. This loose cellular bridge is a transitory feature and in later stages it becomes flattened out between the two trophoblastic layers (Plate 2, figs. 1, 2 and 4; Plate 15, figs. 1, 2 and 3).

The structural equivalent of the placental cone or Träger is very small in the chinchilla; the egg cylinder is attached to the decidua mesometrially by a slender stalk of ectoplacental trophoblast cells which invade the basal decidua at various points but do not present the configuration of the distinctly cone-shaped Träger of the rat or hamster. These cellular protrusions (trophoblastic "villi") serve to anchor the egg cylinder to the uterine decidua at the future site of the allantoic placenta.

Trophoblastic giant cells are not numerous in these early specimens. Whereas in the hamster and rat a discrete zone of giant cells is distinguishable in the region of the placental cone, only a very few trophoblast cells in this area have hypertrophied into phagocytic giant

cells in the thirteen-day chinchilla. Scattered enlarged cells with characteristically swollen nuclei and lightstaining cytoplasm are seen about the inner margin of the decidual cavity and presumably were derived from that portion of the trophoblast antimesometrial to the region of the chorionic cyst.

The uterine lumen at this stage is nearly occluded, appearing only as a small, irregular cavity just above the region of the attachment zone, within which may be seen the degenerating remnants of the uterine epithelium (Plate 1, figs. 1, 3 and 4). In later stages (not dated) this original uterine lumen becomes completely and permanently obliterated.

Directly above the regressing uterine lumen (Plate 1, fig. 2) may be seen a crescent-shaped cleft in the basal decidua which is present in all sections through the loculus. This cleft will in later development become continuous with the newly established uterine lumen and be lined with the same columnar epithelium.

The decidua immediately peripheral to the egg cylinder is converted into a symplasma including extravasated maternal blood. This blood is presumably released from the uterine vessels through the erosive activity of both the yolk sac endoderm and the original extraembryonic trophoblast. Perrotta (20, p. 345) has similarly reported

a striking amount of free maternal blood in the decidual cavity of the porcupine (<u>Erethizon dorsatum</u>), another hystricomorph rodent.

In the thirteen-day stage under consideration (Plate 1, figs. 1, 2, 3 and 4) the egg cylinder has withdrawn somewhat from the border of the decidual cavity although it normally lies quite close to the edge of the symplasma; this is an artefact due to fixation.

The embryo proper at this time consists of a layer of endoderm which is continuous with that of the yolk sac, and the amnicembryonic mass which reposes in the cavity of what will later be the exocoel.

Persistent remnants of uterine glands are seen, particularly in the region of the stratum basale.

Although it was not possible during the course of this study to obtain material in the early implantation stages of development, yet it is evident from the thirteen-day and later embryos that the development of the chinchilla closely parallels that of <u>Cavia</u>. Thus one may tentatively presume that implantation of the blastocyst in the chinchilla is antimesometrial and completely interstitial as in Cavia.

The blastocyst erodes its way through the epithelium of the uterine lumen and comes to lie in a cavity of its own making within the subepithelial mucosa. This

implantation chamber is gradually enlarged by the expanding and erosive trophoblast at the expense of the surrounding decidua to form what is then called the decidual cavity (Plate 14, figs. 1 and 2). With the expansion of the trophoblast, the blastocystic cavity is enlarged and the inner cell mass, located at the mesometrial pole of the blastocyst, proliferates a layer of cells which cap its inner surface (Plate 14, fig. 2). This layer is the presumptive endoderm of the visceral yolk sac which will subsequently acquire an inner lining of splanchnic mesoderm and become the functional yolk sac placenta. If the chinchilla follows the early development of the guinea pig, then a parietal yolk sac layer is never formed and Reichert's membrane soon disappears entirely, resulting in an early, complete inversion of the germ layers (entypy). It may even be that the scattered giant cells of the decidua migrated from the morula and thus no Reichert's membrane could have been established from the start.

This cap of endoderm elongates into a tubular sac extending antimesometrially within the implantation chamber (decidual cavity), later to be seen as the cavity of the symplasma. At the same time the inner cell mass divides and the lower portion, called the amnioembryonic mass, is carried within the sac to the antimesometrial pole of the decidual cavity (Plate 1, fig. 4; Plate 14.

fig. 3). The mesometrial half of the original inner cell mass now represents what in later stages will give rise to the outer ectoplacental trophoblast as well as the inner chorionic ectoderm, between which will form the epamniotic cavity.

Stage 2. Egg Cylinder Stages, Somewhat Later than Thirteen Days

In a series of egg cylinders somewhat more advanced in development (Plate 1, figs. 3 and 4; Plate 2, figs. 1, 2 and 3) several important changes have become evident. By now the egg cylinder is displaced mesometrially, the constricted central portion of the decidual cavity has elongated, imparting a dumbbell shape to the cavity, and the uterine lumen is being reconstructed peripherally. The egg cylinder has broadened, particularly at its mesometrial pole, and the epamniotic cavity is being gradually compressed between the lamina and the ectoplacental trophoblast. In Plate 1, fig. 4 the egg cylinder retains its original slender connection with the basal decidua. but in Plate 2, figs. 1 and 2, depicting a slightly more advanced stage. the ectoplacental trophoblast layer has become flattened and contacts the basal decidua over a much greater area. Note also in these photographs that the decidual cavity seems to surround the egg cylinder completely and fails to present the dumbbell shape previously mentioned. This is because at the particular level from which this section was taken, only the mesometrial part of the cavity was included although the "neck" does appear in sections through the proper level. The yolk sac endoderm at this time is a simple

cuboidal to low columnar epithelium.

The amnioembryonic mass is a flattened cluster of undifferentiated cells in the center of which is a cleft, the incipient amniotic cavity (Plate 2, fig. 3). Although this cavity is here apparent in chinchilla for the first time, Harman and Prickett (16, p. 351-378) state that it is present in the eleven-day blastocyst of the guinea pig, appearing therefore before there is any evidence of the epamniotic cavity. They further state that mesoderm can already be observed at this age; but mesoderm does not become apparent in the chinchilla until much later. Sansom and Hill (24, p. 295-351) a year earlier indicated the epamniotic cavity.

The gestation period for the guinea pig is sixtyeight days (4, p. 308) and that for the chinchilla is 109-111 days. In comparing known developmental stages of the chinchilla with those of the guinea pig provided by Harman and Prickett (16, p. 351-378), the early development of the guinea pig appears to be accelerated over that of the chinchilla by about five to ten days, an observation in agreement with its relatively shorter gestation period.

Stage 3. Early Allantois

The two loculi from the same female upon which this description is based are somewhat advanced over the previous egg cylinder stages. The mesoderm makes its appearance and spreads outward from between the ectoderm and endoderm of the embryonic disc to cover the amniotic ectoderm with somatic mesoderm and line the yolk sac with splanchnic mesoderm. The chorionic ectoderm of the lamina also becomes lined with this simple mesodermal epithelium, and since by definition this mesoderm is somatic, this is an instance of the immediate derivation of somatic mesoderm from previously formed splanchnic mesoderm.

There is as yet no evidence of differentiation among the cells comprising the embryonic disc, but a marked swelling which may be the allantoic anlage is apparent at one edge of the disc (Plate 3, figs. 1 and 2).

The epamniotic cavity is obliterated except for a slit at the junction of the yolk sac and chorion (Plate 3, fig. 3; Plate 15, fig. 1) so that the chorion is now in apposition to the ectoplacental trophoblast (Plate 2, fig. 4; Plate 3, fig. 1). Maternal blood is absent from the persisting epamniotic slit and does not envelop the embryonic vesicle as it did in some of the earlier stage embryos.

The embryonic vesicle has both filled the upper

chamber of the decidual cavity and extended through the narrow "neck" into the expanding lower chamber. This lower chamber (antimesometrial) of the decidual cavity is relatively larger than the mesometrial portion, and is partially filled by a very loose network of capillaries and cells of the symplasma. The symplasma bounds the decidual cavity and is continuous with the necrotic tissue of the decidua basalis in the zone of junction.

The uterine lumen is now completely re-established both peripherally and antimesometrially; it is lined by the characteristic simple columnar epithelium. Mesometrially the crescent-shaped cavity (in section), seen forming in the decidua basalis of earlier stages, is now more extensive and completely separates the placental disc from the overlying uterine tissues (Plate 2, fig. 4; Plate 3, fig. 1). This hemispherical cavity is not continuous with either the uterine lumen or the decidual cavity at this time; neither is it lined by an epithelium. The lumen of this cavity is filled with fluid in which are suspended scattered cells and cell remnants.

Both the hemispherical cavity above and the antimesometrial portion of the decidual cavity below have so extended and approximated their peripheral limits as to delimit medially a thick-walled cup of decidual tissue which forms the boundaries of the "neck" and upper chamber

of the decidual cavity. It will be noted in Plate 3, fig. 1 that the thick wall of this cup is permeated with blood vessels which pass through the narrow ring of tissue by which, at this stage, the cup is attached to the more peripheral decidua capsularis.

The thick, highly vascular wall of the decidual cup together with its slender, circular connection with the uterine wall is suggestive of the mesoplacentarium of another hystricoid rodent, the agouti (<u>Dasyprocta azarae</u>), described by Strahl (26, p. 524-528) and Becher (6, p. 337-364; 7, p. 439-454). In the agouti the placenta lies free, connected to the uterine wall by means of a thin decidual membrane only. This membrane in the agouti attaches around the equator of the globe-shaped placenta and accommodates the maternal arteries and veins which supply the placenta. Thus both the chinchilla and agouti share in this structural feature.

The elaboration of the deciduae in the guinea pig (18, Plate 17) and the chinchilla is accomplished by a splitting of the endometrium which establishes the new uterine lumen. In these animals the original uterine lumen becomes obliterated by the expanding decidua, as previously stated for the chinchilla. The new lumen appears secondarily both antimesometrially and peripherally as a cleft (in section) in the uterine stroma. This

establishes both a decidua parietalis peripherally, and a decidua capsularis reflected over the conceptus.

Stage 4. Later Allantois, 25-30 Days Gestation

Further development of the allantoic primordium into a spheroidal mass (Plate 3, fig. 4), and the early differentiation of the embryonic disc mark the most significant advances in the embryo at this stage. There are four or five pairs of somites, neurulation has begun, and the omphalomesenteric blood vessels have established communication between embryo and yolk sac. The anterior portion of the embryo is defined by the undercutting of the amnion. The ectodermal and mesodermal layers of the amnion are loosely adherent.

The mesoderm of the amnion is continuous posteriorly with that of the allantoic primordium, which has by now expanded into the exocoel as a loose mass composed of a scant matrix of mesenchyme into which extend a series of irregular blind pouches formed by the multiple invagination of the allantoic epithelium (Plate 3, fig. 4). There is a transition from the low cuboidal surface epithelium of the allantoic mass to a columnar epithelium which makes up the blind limits of the invaginations. There is little evidence of blood cell or blood vessel formation in this allantois, and no indication of any endodermal yolk sac element.

The bilaminar yolk sac presents no special structural

changes over that of the preceding stage. A few scattered blood islands are seen in its splanchnic mesoderm, more numerous in the region immediately adjacent to the embryo.

The ectoplacental trophoblast is now represented by a few scattered cells in the adjoining necrotic zone (maternal), thereby exposing the chorionic trophoblast of the lamina to the extravasating maternal blood. This blood bathes the mesometrial limits of the chorionic ectoderm and fills the epamniotic cavity which is again re-established. That this blood invasion is a normal occurrence is evident from those specimens which were not injected intravascularly with fixative and therefore subjected to no artificial breaks in the blood barriers. See Plate 4, figs. 1 and 4.

The chorionic trophoblast has undergone extensive proliferation, such as to divide the exocoel into an upper and lower chamber, leaving only a small intercommunication (Plate 4, figs. 2 and 3). The medial expansion of the decidual cup appears to have been a factor in establishing this constriction. The lining of the upper exocoel is chorionic mesoderm (Plate 4, fig. 1) and extends as a thin layer of lightly staining cells. The upper exocoel represents what in later stages of the placenta is called the cavity of the subplacenta (cavity of accessory placenta). At this stage then, the subplacenta (accessory placenta) is composed of the lamina (chorionic ectoderm plus lining somatic mesoderm). Later, this structure will include a sparse allantoic vascular supply; therefore the definitive subplacenta is entirely of fetal origin and will be found in a central excavation on the mesometrial aspect of the placental disc (Plate 6, fig. 4; Plate 17, fig. 1).

In near term placentae the subplacental cavity becomes much reduced in size, and may even disappear along with the subplacental tissues (Plate 7, figs. 1, 2 and 3).

A noticeable increase in the size of the uterus and its associated blood vessels has taken place in accommodation of the growing conceptus. The uterine wall has become attenuated and the cellular hypertrophy of the earlier decidual reaction in the endometrium is giving way to layers of flattened or fusiform cells.

The peripheral areas of the decidua capsularis have a few irregular clefts when viewed in section. These clefts separate folds of tissue from the main part of the capsularis giving it a lamellar appearance, but these folds are all in continuity.

Stage 5. Thirty Days Gestation (Rancher's Record)

Slightly older than the preceding, this embryo has five or six pairs of somites, a distinct neural tube with brain vesicles, and a two-chambered heart. Although the yolk sac endoderm is complete and fully lined with mesoderm, yet angiogenesis is restricted to the antimesometrial half of the yolk sac. The omphalomesenteric arteries are well developed and open into the dorsal aortae (Plate 5, fig. 4).

The somatic mesoderm of the amnion bears many hollow buds or vesicles which are covered with a discontinuous layer of light-staining globules. Most of these vesicles are empty, although occasionally one may find a few contained elements which are interpreted to be primordial blood cells (Plate 5, fig. 3). In advanced stages of development, no vascular elements of the amnion could be found; thus the definitive amnion is a smooth, avascular membrane.

Angiogenesis is considered more a property of splanchnic rather than somatic mesoderm. Although the primate amnion has been classically described as avascular and lacking in angiogenic potentialities, blood formation by first intention has been reported in amniotic mesoderm by Noback (20, p. 553-567) in the baboon,

<u>Papio papio</u>, and by Fischel (13) in man. In this connection it is of interest to note that a <u>secondary</u> vascularization of the amnion occurs after its fusion with the allantois in artiodactyls, perissodactyls, and in carnivores (18, p. 152-153).

Unfortunately important sections in the hind gut region were lost during preparation, making it impossible to ascertain the embryonic connection of the allantois in this embryo. However, several elements taken to be portions of the allantois were found near the amnion in the caudal region of the embryo. One such element is that figured in Plate 5, figs. 1 and 2. This item appears to be composed of allantoic mesoderm enclosing a small blood island and a core of cuboidal cells. These latter cells are arranged in a circle and suggest a section through what may be either the endodermal allantoic or yolk sac diverticulum.

The chorionic ectoderm has proliferated many trophoblastic lamellae, forming a trabecular network, the interstices of which are packed with maternal blood (Plate 4, fig. 3). Maternal blood also fills the lateral remnants of the epamniotic cavity (Plate 4, fig. 4). In this same figure one can see that the yolk sac endoderm continues mesometrially from the chorion-yolk sac junction and does so in association with the attenuated ectoplacental

trophoblast. Further mesometrially the yolk sac endoderm and ectoplacental trophoblast lose their identity as they merge with the necrotic zone.

The cavity of the developing subplacenta is now larger and possesses a distinct lining of chorionic mesoderm which is quite thick in the slender neck communicating with the lower exocoel (Plate 15, fig. 3; Plate 16, fig. 1). This chorionic mesoderm is a very loose basophilic reticulum, the cells of which resemble mesenchymal cells or fibroblasts.

The symplasma which lines the decidual cavity is now deteriorated extensively, particularly in its antimesometrial limits where little tissue remains to separate the decidual cavity from the newly established uterine lumen.

Many small loops of decidual blood vessels, which apparently are yet unaffected by the process of necrosis, lie free in the decidual cavity (Plate 6, fig. 1).

Stage 6. Embryo, 6 mm Crown-Rump

The yolk sac directly beneath the embryo has become constricted into a stalk which contains the vitelline vessels serving the embryo. The splanchnic mesoderm of the yolk sac has elaborated blood channels mesometrially as far as the chorion-yolk sac junction. Here the yolk sac is thrown into numerous folds which later form the digitiform sacculations intimately associated but not fused with the placental disc. No sinus terminalis is as yet established.

The allantoic mesoderm has crossed the exocoel to spread over the chorion as well as insinuate itself into the canal leading to the subplacental cavity (Plate 6, fig. 2). Since it has not passed along the full extent of this canal, no allantoic mesoderm is in the subplacental cavity. At certain points on the embryonic surface of the chorion, especially the central region, the allantoic blood vessels have begun to penetrate into the overlying spongy zone (trophospongium) composed of syncytial chorionic ectoderm, the interstices of which are permeated with free maternal blood (Plate 6, figs. 2 and 3; Plate 16, figs. 2 and 3). This penetration of the trophospongium by the allantoic blood vessels presages the development of the placental exchange area (labyrinthine zone) and established the primary relationship between the embryonic and maternal blood streams. Many nucleated red blood cells are present in the allantoic blood vessels.

A layer of giant cells is identified peripherally in the zone of junction between the trophospongium and the necrotic zone. More centrally in the junctional zone however, only a few scattered giant cells are found.

The symplasma remains as a continuous encircling area, and the decidua capsularis persists unbroken. The decidual cells of the capsularis are, for the most part, larger than those of the basalis; both layers contain well-defined vascular channels.

Stage 7. Embryo, 24 mm Crown-Rump; 58 Days Gestation (Rancher's Record)

The allantoic mesoderm has penetrated the chorionic layer and so extensively permeated the trophospongium that the hemochorial placental condition has been established. As the major fetal allantoic vessels branch within the trophospongium they elaborate a series of capillary beds which are destined to become the future placental exchange areas or labyrinthine zones. These fetal capillaries radiate from the central vessels, and many such clusters provide the lobate composition of the definitive placenta.

The trophospongium forms a cortex surrounding the labyrinthine zones and continues into the latter as attenuated trophoblastic tubules bearing maternal blood. The central zone of the labyrinth contains a variable number of maternal arterial channels and fetal veins. Situated at the junction of the labyrinth and trophospongium are the smaller fetal arteries. In comparison with the nearterm placenta the labyrinth appears to be less expanded and the maternal and fetal blood streams pass in rather tortuous adjacent paths.

The subplacenta is a comparatively extensive zone and consists, for the most part, of chorionic ectodermal cells of uniformly small size. These cells occur in dense

nests or strands held in a matrix of loose chorionic mesoderm, all of which is vascularized rather irregularly and sparsely by the allantoic mesoderm. Only a few larger maternal vessels are seen peripheral to the subplacenta; these are en route elsewhere and apparently are not involved in the blood supply of this structure.

In some preparations there are areas of chorionic ectoderm both mesometrial and peripheral to the subplacental area proper, which consist of cells in continuity with the subplacenta. But here the nuclei stain a darker blue and the whole tissue is permeated by maternal blood so that it resembles in pattern the trophospongium. This material is in turn continuous with and grades into the true trophospongium of the adjacent lobes.

The placental disc has so expanded peripherally that the yolk sac, which originally made contact with the disc around its margin, is now carried against the fetal aspect of this disc. From the new peripheral limits of the yolk sac, a layer of giant cells takes origin and extends peripherally and mesometrially around the margin of the placental disc. This giant cell layer is composed of cells of markedly different diameters and is of variable thickness (Plate 9, fig. 3). Tracing the giant cell layer to the mesometrial aspect of the placental disc, one can observe large nests of these cells just outside the

trophospongium (Plate 8, figs. 1 and 2). The nuclei are variously shaped and some are extremely large. Other cells may have two nuclei, rarely three or four, and sometimes the nucleolus is very prominent. These cells appear to be transformed ectoplacental trophoblast cells, including those of the placental cone area, all of which have greatly increased in size. Transitional stages in the transformation of chorionic ectodermal cells into these giant cells can be seen in many places.

The placental disc, with the adjacent basal and capsular decidua, is now extensively undercut, leaving only a narrow communication between the placental base and uterine wall. In the space between the placenta and uterus, vascularized loops of the plicated yolk sac have insinuated themselves (Plate 16, fig. 3; Plate 17, fig. 1).

The decidua capsularis is now broken through antimesometrially and the uterine epithelium is re-established on both its inner and outer aspects. Regenerated uterine epithelium is found to be discontinuous over the surface of the capsularis and basalis in the region mesometrial to the placental disc. In some areas on the decidua capsularis the uterine epithelium has become a high columnar, pseudo-stratified epithelium.

In the decidua basalis there are many uterine vessels which in cross-section bear greatly hypertrophied

endothelial lining cells. In a few sections these cells are somewhat reminiscent of endovascular plasmodial cells; however, these do not occlude the vascular lumen to any marked extent.

The extensive necrotic region in the zone of junction is, for the most part, composed of tissue detritus and light-staining residues.

Stage 8. Embryo, <u>60 mm Crown-Rump;</u> <u>75 Days</u> <u>Gestation</u>

Both the choricallantoic placenta and yolk sac have already attained definitive size and differentiation in the 60 mm embryo stage. The labyrinthine zones of the lobes have fully expanded so that the routes of blood flow have straightened appreciably, and no longer follow the tortuous paths characteristic of earlier stages.

The somatic mesoderm of the chorion is a well-defined layer of cells, particularly in the central portion of the placental disc, within the circle outlined by the sinus terminalis. In this same area the amnion has become adherent to the chorionic mesoderm; the two may be readily distinguished (Plate 9, fig. 1). The amniotic ectoderm consists of a simple low cuboidal to squamous epithelium. The dark-staining nuclei of these ectodermal cells are ovoid in shape and occupy a central position in the cell.

The nonvascularized portion of the yolk sac, adherent to the peripheral fetal aspect of the placental disc, retains continuity with the giant cell layer. As one moves toward the center of the fetal aspect of the placental disc the endodermal epithelium, composed of low columnar to cuboidal cells, is thrown into simple folds. It makes an abrupt turn and doubles back upon itself at the site of the sinus terminalis (Plate 8, figs. 3 and 4).

This vascular layer of the yolk sac then pursues a course which roughly follows the contours of the placental disc mesometrially. The yolk sac becomes more folded en route so that numerous villi are established, some branched, and all with a good blood supply (Plate 9, fig. 4). The endodermal cells of the villi are high columnar and bear on their free borders globules suggesting secretory activity.

The yolk sac continues mesometrially to the region of placental undercutting. Where it reaches the necrotic zone, it recurves and passes antimesometrially with numerous saccular diverticula insinuating into the crevices between the folds of the capsularis. Some of these sacculations extend into the irregularities on the peripheral surface of the placental disc, others into the reconstituted uterine lumen.

The subplacenta is a massive structure containing small remnants of the former subplacental cavity (Plate 6, fig. 4). Its composition and vascular supply is like that described earlier.

The necrotic zone is more extensive than in earlier stages; necrosis has also involved the thickened basal decidua and the peripheral decidua capsularis.

<u>Morphology of the Definitive</u> <u>Choricallantoic Placenta</u>

The definitive placenta of the chinchilla is oval or discoid in shape, and measures about 25 mm to 30 mm in diameter. Viewed from the fetal aspect, it may appear slightly lobate, and is broadly undercut on the mesometrial aspect (Plate 17, fig. 1). The reflection of the visceral yolk sac over nearly the entire free surface of the placenta was previously mentioned.

In the labyrinthine zone one can clearly discern, under oil immersion, the extensive cytoplasm of the trophoblast cells enclosing their large spheroidal nuclei. These trophoblast cells form tubes, the walls of which are one cell thick. The cytoplasm becomes attenuated between cells and forms a thin membrane in contact with the adjoining fetal capillary tubes. The diameters of the trophoblast tubes vary from two to four times that of the fetal capillaries (Plate 13, fig. 5).

The fetal capillary tubes are composed of a continuous endothelium, the nuclei of which are ovoid and stain darker than the trophoblast nuclei. Thus the two types of nuclei can be readily distinguished. The fetal capillaries are very uniform in diameter, usually about 7 microns. Since the walls of both the endothelial tubes and the trophoblast tubes are intact and continuous, the

mature placental labyrinth is hemochorial. If there are any areas in which the trophoblastic cells are discontinuous, then such a hemoendothelial condition is left for electron microscopic examinations to establish.

In general, both the endothelial and trophoblast tubes radiate as unbranched channels from the center to the periphery of the lobes (Plate 13, figs. 1, 2 and 4). There are however, observable instances in which transverse anastomoses occur, both among trophoblastic tubes as well as among the endothelial tubes.

In transverse section the trophoblast tubes and the smaller endothelial tubes are so arranged that they approach the "star" pattern of a rete mirabile (25, p. 99). Thus the larger trophoblast tube has arranged about its circumference a variable number of the smaller endothelial tubes. These same smaller tubes are involved again in other rings of endothelial tubes, which, in turn, surround other trophoblast tubes. This physical arrangement makes for the greatest possible area of exchange.

The trophospongium is a portion of the original chorionic ectoderm which, while it did become extensively permeated by free maternal blood, did not become further attenuated by an ingrowth of the allantoic mesoderm as in the case of the labyrinth.

The allantoic mesoderm begins at the junction of

trophospongium and labyrinth. Here the fetal arterial channels, which have penetrated the trophospongium, thin out and subdivide into the endothelial tubes. Centrally the labyrinth ends where the endothelial tubes anastomose and immediately empty into a variable number of fetal venous channels.

The decidua basalis which enclosed the developing fetal placenta and constitutes the maternal placenta, is thickened into a transverse "saddle" of tissue continuous with the peripheral mesoplacentarium, which in turn connects with the uterine wall. Mesometrially a stalk or pedicle of decidual tissue serves to connect the placental disc with the uterus. The definitive placental connection to the uterus therefore occurs at three points: the two attachment regions of the mesoplacentarium, one on either side of the placental disc, and the basal pedicle, located mesometrially. Except for the basal pedicle, the means of placental attachment to the uterus in the chinchilla closely corresponds to that of the agouti.

Morphology of the Definitive Yolk Sac

The yolk sac is attached to the placenta along a circular line midway between the margin of the placental disc and the centrally located umbilical cord. It is never attached to the placenta elsewhere although the two may be in very close apposition. The attachment of the yolk sac to the placental disc coincides with the sinus terminalis. The placental surface between the sinus terminalis and the insertion of the umbilical cord is not covered by yolk sac; only the amnion intimately contacts this area of the placental disc.

At the periphery of the placental disc the yolk sac is thrown into numerous vascularized loops or sacculations which extend into crevices at the placental margin and on the mesometrial aspect of the placenta. Beyond the margin of the placental disc, where these sacculations take their origin, the remainder of the yolk sac extends antimesometrially, surrounds the fetus, and joins the vitelline stalk. The yolk sac also may extend beyond the limits of the loculus into the interlocular regions of the uterus. It may even overlap the yolk sac of the next fetus if there are several loculi in the uterine horn.

The Umbilical Cord

The definitive umbilical cord of the chinchilla is about 20 mm in length. In cross section there are five major blood vessels: two umbilical arteries of nearly equal size, one larger umbilical vein, and a small vitelline artery and vein (Plate 12, figs. 1 and 2). Serial sections through the abdominal wall of the fetus indicate that it is the right, rather than the left, umbilical vein which persists in the chinchilla.

In addition there are smaller vessels, containing blood cells, scattered in the substance of the cord. These are presumed to be the vascular elements of the umbilical cord stroma (Plate 12, fig. 2). These smaller blood vessels are supplied and drained by the major allantoic arteries and vein. This interpretation of blood vessels in the cord stroma of the chinchilla gains support from observations in other forms such as most ungulates and the Cetacea, in which the entire umbilical cord stroma contains a rich supply of small blood vessels and capillaries; in contrast, the umbilical cord stroma in man and monkeys is avascular (14, p. 90). It has been suggested that the stromal blood vessels of the umbilical cord and the amniotic blood vessels may be involved in amniotic fluid transfer in ungulates and cetaceans (14, p. 90). The epithelium of the yolk sac vesicle was traced in the embryo from the gut into the proximal end of the cord; beyond this level only discontinuous remnants of the vesicle epithelium could be identified in placentae of one month or more gestation.

There is also present in the umbilical cord an element which has been identified as the allantoic vesicle. This structure, which was seen in all sections of the cord examined (except those near the placental surface) is composed of simple cuboidal epithelium, its lumen filled with a light-staining amorphous material. Closely associated with this vesicle are many capillaries.

The mucous connective tissue of the cord is bounded peripherally by a simple squamous epithelium to which the amnion is closely adherent. The mucous connective tissue of the early gestation cord continues into the substance of the placenta, investing the allantoic vessels along their course to the exchange areas. In late gestation however, this connective tissue disappears from the deeper allantoic vessels remaining only with the more superficial vessels.

The umbilical cord is usually attached centrally on the fetal aspect of the placenta, rarely eccentrically.

The yolk sac blood vessels diverge from the umbilical cord shortly before it reaches the placental disc, and

continue independently toward the sinus terminalis as the vitelline stalk. The vitelline stalk leaves the umbilical cord at an acute angle and forms the hypotenuse of a triangle, the base and side of which are the placental surface and the umbilical cord respectively.

II. THE VASCULAR PATTERNS IN THE DEFINITIVE VITELLINE AND ALLANTOIC PLACENTAE

The Allantoic Placenta

A. <u>Maternal</u>. At regular intervals the uterine arteries give off branches which pass through the mesometrium to the uterus. These so-called primary segmental branches of the uterine artery (22, p. 6-7) usually group together in fours to become associated with a similar tributary of the uterine vein, likewise passing through the mesometrium (Plate 10, figs. 1 and 2).

Where these segmental arteries reach the body of the uterus they enter and pass between the longitudinal and circular muscle coats to course peripherally around the loculus as the circumferential arteries (22, p. 6-7). The arteries do not form a "mesometrial triangle" in the uterine stroma as described in the rat by Young (27, p. 297-298).

About one-half of the way around the sides of the loculus the majority of the circumferential arteries, here greatly subdivided into smaller vessels, pass into the mesoplacentarium, within whose substance they become highly coiled. Beyond this area they collect into several large arterial sinuses to enter the placental disc through the junctional zone (Plate 11, fig. 4). Once within the placenta these large sinuses branch into the central zones of the placental lobes as the maternal arterial channels.

The vascular knot of the hamster uterus is thought to function in the reduction of blood pressure and pulse pressure differential (20, p. 583). A similar function may presumably be attributed to the plexus of vascular channels in the mesoplacentarium of the chinchilla placenta.

As the maternal arterial blood flows outward from the central zones of the lobes and thence through the trophoblastic tubes, it bathes the fetal endothelial tubes carrying fetal blood en route to the central zones. The maternal blood filters out through the trophospongium toward the periphery of the placenta where it is collected in a network of maternal venous channels located just beneath the placental surface (Plate 11, fig. 3; Plate 12, fig. 3). These channels convey the maternal blood toward the mesometrial aspect of the placenta, where the channels eventually collect into one or two large mesometrially located veins draining out through the basal pedicle of the placental disc to enter the mesometrium (Plate 10, figs. 3 and 4; Plate 11, fig. 2).

B. <u>Fetal</u>. The two umbilical arteries and single umbilical vein penetrate centrally into the embryonic aspect of the chorioallantoic placenta. Just beyond the point where the

umbilical arteries enter the placental disc a single, short, transverse anastomosis is established between them. This connection has also been observed in the human placenta by Bacsich and Smout (5, p. 363) who believe this union allows an equal distribution of blood and regulates pressure in the placenta, thus serving as a buffer system against the effects of uterine contraction.

Within the placenta, between the trophospongium and labyrinthine zones, the umbilical arteries subdivide into a series of finer ramifications which encircle the individual placental lobes. Then, after subdivision into capillaries, they pass centrally through the labyrinth toward the central zones as the fetal endothelial tubes. These capillaries are in apposition to the trophoblastic tubes conveying blood from the maternal arterial channels of the central zones toward the peripherally located trophospongium. Thus the maternal and fetal blood streams course in opposite directions within the labyrinthine zone, an adaptation allowing for an efficient exchange gradient between fetal and maternal circulations, as first emphasized by Mossmann (17, p. 433-497) in the rabbit.

Where the endothelial tubes reach the central zone of the lobe they coalesce into a number of larger central veins within this zone (Plate 13, fig. 3). These larger venous channels from the several placental lobes unite

in turn to form ultimately, the single umbilical vein (Plate 12, fig. 4).

Vitelline Placenta

A. <u>Maternal</u>. Since the yolk sac lies in intimate apposition to the extravasated maternal blood of the symplasma zone throughout gestation, all of the finer branches of the maternal circumferential arteries and veins in the symplasma zone constitute the maternal vascular supply to the vitelline placenta.

B. <u>Fetal</u>. The single artery which supplies the yolk sac takes its origin in the splanchnic mesoderm of the embryonic gut, passing ventrally to leave the body and enter the umbilical cord. It courses distally for about one-half to two-thirds the length of the cord and then separates from it to enter the vitelline stalk in company with the vitelline vein.

The vitelline stalk angles sharply away from the cord and its two blood vessels reach the embryonic surface of the placenta somewhere near the periphery of the placental disc. After reaching the placental surface the yolk sac artery more frequently than not, divides into two branches which encircle the placental surface as the sinus terminalis. These two branches do not meet on the opposite side of the placenta; they are however, usually connected by means of a multiple anastomosis of their finer branches in this region. The arterial branches extending from the sinus terminalis are directed peripherad over the margin of the placental disc and form a network of finer arterioles and capillaries within the substance of the yolk sac.

Although the vitelline stalk and the sinus terminalis are located at the periphery of the developing placental disc, their definitive position is about halfway between the placental border and the centrally located umbilical cord. This position is the result of the subsequent increase in size of the placental disc.

The network of venules which drain the yolk sac are gradually collected into several veins which in turn unite to form the vitelline vein near the site where the vitelline artery joins the sinus terminalis. There is thus a single vitelline vein coursing parallel with the yolk sac artery in the vitelline stalk. Where the yolk stalk unites with the allantoic cord, the vitelline blood vessels continue as an integral part of the umbilical cord to the fetus.

SUMMARY

- Both the pattern of implantation (antimesometrial) and early development of the egg cylinder of the chinchilla are similar to those of <u>Cavia</u>, but the development is slower.
- A reduced Träger is present, giant cells are scarce, no parietal yolk sac was found, and Reichert's membrane (if ever present) disappears early.
- 3. The choricallantoic placenta is located mesometrially in a cup of maternal decidual tissue investing all but the embryonic aspect of the disc. This cup becomes separated from the uterine stroma mesometrially and peripherally except for two bands of tissue conveying the maternal blood channels to and from the placenta. These bands are comparable to the mesoplacentarium of the agouti placenta.
- 4. A prominent subplacenta is formed early in the development of the chorioallantoic placenta and persists during the greater part of gestation. Eventually it becomes reduced in size and usually disappears entirely in the near term placenta.
- 5. Internally the choricallantois is an assembly of lobes, the areas of exchange between maternal and fetal blood streams. Each lobe consists of a central zone, a surrounding labyrinthine zone of trophoblastic

tubes and fetal capillaries, and an investing zone of trophospongium.

- 6. Maternal blood from the central zone flows out through the radiating trophoblastic tubes of the labyrinthine zone and is collected in the sinuses of the trophospongium. Fetal blood, passing inward from the peripherally located vessels, flows through the endothelial tubes of the labyrinthine zone and is drained by the fetal venous channels in the central zone of the lobe.
- 7. Since the fetal and maternal blood streams are separated by trophoblast and endothelium, both of fetal origin, this placenta is histologically hemochorial.
- 8. The highly vascularized visceral yolk sac persists throughout gestation. Fingerlike diverticula develop from the yolk sac in the region of the sinus terminalis and become incorporated into clefts of both the placental disc and the mesoplacentarium.
- 9. Blood vessels reach the yolk sac via the umbilical cord and yolk stalk. The vitelline artery bifurcates to form a circular sinus terminalis from which radiate finer channels to supply the yolk sac capillary network. Returning venous blood collects into the vitelline vein at the point where the yolk stalk reaches the placental disc.

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Plate 1*

- Fig. 1. Cross section through entire loculus of 13 days gestation. The egg cylinder lies in the upper chamber of the implantation cavity; the lower chamber is filled with maternal blood. 13X. Hematoxylin and eosin. (309-LB)
- Fig. 2. Same as fig. 1 but higher magnification to show the detail of the egg cylinder. Note the irregular column of cells extending from the chorionic lamina across the epamniotic cavity to the ectoplacental trophoblast. The amnicembryonic mass is visible at the lower pole of the egg cylinder. 50X. Hematoxylin and ecsin.
- Fig. 3. Cross section through a slightly older loculus. The implantation cavity has enlarged and the uterine lumen may be seen reappearing along the right hand side. 13X. Hematoxylin and eosin.
- Fig. 4. Higher magnification of the egg cylinder seen in fig. 3. The degenerating original uterine lumen may be seen as a clear oval immediately above the point of attachment of the egg cylinder. Note the loose network of maternal symplasma tissue lateral to the egg cylinder. 40X. Hematoxylin and eosin.

* - In all plates, unless otherwise indicated, the preparations are oriented so that the mesometrium is toward the top of the page.

- Fig. 1. Cross section through another loculus from the same female as Plate 1, fig. 4. The mesometrium is toward the left of the photo. The darkstaining masses peripheral to the egg cylinder are maternal blood. The light spot in the amnioembryonic mass is the first indication of the amniotic cavity. 20X. Hematoxylin and eosin.
- Fig. 2. The same preparation as fig. 1 at higher magnification to reveal the much reduced trophoblast of the placental "cone". The epamniotic cavity is visible as a discontinuous crescent between the chorionic lamina and the placental "cone" (ectoplacental trophoblast). 50X. Hematoxylin and eosin.
- Fig. 3. Amnioembryonic mass of an older egg cylinder showing the forming of the amniotic cavity by cavitation in the center of the mass. The mesometrium is toward the left. 100X. Hematoxylin and eosin.
- Fig. 4. Cross section through entire loculus of 20-25 days gestation. The basal cavity may be seen directly above the thick-walled decidual cup in which lies the egg cylinder. The dark-staining areas in the walls of the cup are maternal vessels filled with blood. About 4[±]/₂X. Masson III.

- Fig. 1. Higher magnification of a portion of the loculus seen in Plate 2, fig. 4. The tortuously coiled maternal blood vessels (dark-staining areas) may be seen entering the decidual cup. Masson's trichrome stain. 13 X.
- Fig. 2. Amnicembryonic mass of the loculus seen in Plate 3, fig. 1. The amnictic cavity is evident, separating the amnion from the embryonic disc. The bulge at one edge of the embryonic mass is presumed to be the allantoic anlage. Masson's trichrome stain. 40 X.
- Fig. 3. Junction of the chorion (right) with the yolk sac (left). A portion of the epamniotic cavity may be seen between the two structures above the point where they join. Masson's trichrome stain. 400 X.
- Fig. 4. Embryo of 25 days gestation showing the vesicular allantois with an epithelial invagination opening to the surface (arrow). Note the "loose" texture of the amnion. The anterior portion of the embryo does not show because of the oblique plane of section. Masson's trichrome stain. 40 X.

- Fig. 1. Developing choricallantoic placenta of twentyfive days gestation. The subplacental cavity is apparent in the center of the photograph. The dark areas are maternal blood which fills the trophoblastic interstices. 316A. Masson's trichrome stain. 13 X.
- Fig. 2. Developing choricallantoic placenta of thirty days gestation. The cavity of the subplacenta is still continuous with the exocoel by means of a slender canal. 210B. Hematoxylin and eosin. 15 X.
- Fig. 3. Higher magnification of the canal between the subplacental cavity and the exocoel. The dense, dark-staining tissue is chorionic ectoderm; the lighter, more loosely organized tissue delimiting the canal is chorionic mesoderm. Meso-metrium to the right. 210B. Hematoxylin and eosin. 40 X.
- Fig. 4. Junction of the chorionic ectoderm and ectoplacental trophoblast with the yolk sac. A small portion of the epamniotic cavity is still visible near the junction. The major part of this cavity has been obliterated by invading trophoblast and extravasated maternal blood. The endoderm of the yolk sac (arrow) continues mesometrially for a short distance in apposition to the ectoplacental trophoblast. 210B. Hematoxylin and eosin. 100 X.

- Fig. 1. An isolated fragment presumed to be part of the allantois, the major portion of which is missing in this specimen. Parts of the embryo, amnion and yolk sac may be seen in the lower left corner. 210B. Hematoxylin and eosin. 100 X.
- Fig. 2. Enlargement of the allantoic fragment seen in Fig. 1. In this illustration the endodermal vesicle is apparent (arrow). 210B. Hematoxylin and eosin. 400 X.
- Fig. 3. A section of the amnion of a thirty-day-old embryo. The somatic mesoderm layer to the left is thrown into folds, one of which contains what is presumed to be a developing blood cell. The embryo is to the right. 210 B. Hematoxylin and eosin. 400 X.
- Fig. 4. Transverse section through an embryo of thirty days gestation. One of the omphalomesenteric arteries (arrow) may be seen joining one of the branches of the dorsal aorta. 210B. Hematoxylin and eosin. 200 X.

- Fig. 1. A portion of a section through a loculus of 30 days gestation to show the extensive deterioration of the symplasma surrounding the conceptus. The blood vessels of the symplasma, which have not yet been affected by necrosis, appear as isolated rings (actually loops). Part of the embryo may be seen in the upper left corner. Mesometrium to the left. 210 B. Hematoxylin and eosin. 50 X.
- Fig. 2. Allantoic splanchnic mesoderm (arrow) penetrating the embryonic aspect of the placenta. To either side is trophospongium filled with maternal blood. 169 B. Hematoxylin and eosin. 30 X.
- Fig. 3. A different area of the same section seen in Fig. 2. Here the splanchnic mesoderm from the allantois (arrows) is invading the maternal blood lacunae of the trophospongium. A portion of the visceral yolk sac may be seen across the bottom of the photograph. 169 B. Hematoxylin and eosin. 100 X.
- Fig. 4. Cross section through the placenta from a loculus containing a 40 mm fetus. The subplacenta is seen as a vacuolated structure occupying a central position in the photograph. The mesoplacentarium (arrows) flanks the placental disc. Mesometrium to the right. 286-1. Hematoxylin and eosin. About 4 X.

Plate 7.

- Fig. 1. Cross section through a loculus from which a 26 mm fetus was removed. A rather large subplacental cavity still exists and the mesoplacentarium is extensive. Mesometrium to the left. 307-A. Masson's trichrome stain. About 2 X.
- Fig. 2. A preparation similar to that shown in Fig. 1, but the fetus in this loculus measured 40 mm. The choricallantoic placenta now shows the characteristic internal lobes and the subplacenta is a comparatively large structure but its cavity is reduced in size. Mesometrium to the left. 286. Masson's trichrome stain. About 2 X.
- Fig. 3. Cross section through a near term loculus which contained a 60 mm fetus. The subplacenta has completely disappeared and the mesoplacentarium is considerably reduced; its points of attachment to the placental disc are not apparent in this section. 213. Masson's trichrome stain. About 5 X.

- Figs. 1 and 2. Clusters of giant cells near the junctional zone of the choricallantoic placenta. 213, 303-2. Masson's trichrome stain. 200 X.
- Fig. 3. Point of attachment of the yolk sac to the choricallantoic placenta. The large blood vessel in the yolk sac near the point of attachment is the sinus terminalis. Meso-metrium to the left. C-69. Hematoxylin and eosin. 100 X.

Fig. 4. Point of attachment of the yolk sac to the choricallantoic placenta. In the region where this section was made the sinus terminalis is incomplete, thus accounting for its absence in the photograph. Meso-metrium to the left. 286. Masson's tri-chrome stain. 100 X.

- Fig. 1. Section through the embryonic aspect of the choricallantoic placenta central to the sinus terminalis. The light-staining, loosely organized choricnic mesoderm is a rather thick zone appearing somewhat folded. The dark region to the extreme left is trophospongium. C-69. Masson's trichrome stain. 150 X.
- Fig. 2. Section through the embryonic aspect of the choricallantoic placenta peripheral to the sinus terminalis. The amnion is apparent as a thin, dark membrane to the right. The thick yolk sac is seen between the amnion and the placental disc. Mesometrium to the left. 307A. Masson's trichrome stain. 100 X.
- Fig. 3. Region of intergradation between yolk sac endoderm and giant cells (arrow) on the embryonic aspect of the placental disc. The area shown is just peripheral to the sinus terminalis. C-69. Masson's trichrome stain. 100 X.
- Fig. 4. Yolk sac villi near the peripheral margin of the placental disc. The areas indicated by arrows are blood vessels of the yolk sac. C-69. Hema-toxylin and eosin. 100 X.

- Fig. 1. Nonpregnant uterine horn in which the arteries have been injected with latex. Entire preparation cleared in glycerine. 2 X.
- Fig. 2. Nonpregnant uterine horn from a female in estrus. The veins have been injected with latex, and the entire preparation cleared in glycerine. 2 X.
- Fig. 3. Latex cast of the maternal arterial (light) and venous (dark) blood vessels of the placenta to show their continuity with the maternal vessels in the mesometrium. The placenta is viewed from the maternal aspect with the mesometrial vessels pulled toward the top of the photo. The masses of light-colored vessel casts on either side of the placental disc represent the maternal arterial vessels which are coiled in the mesoplacentarium. 281-1R. 2 X.
- Fig. 4. Vinyl acetate cast of the fetal arteries and maternal veins in the placenta. The dark vessel casts toward the upper part of the photo represent the maternal veins in the mesometrium and part of the placenta. The lighter vessel casts below are a portion of the fetal arterial vessels in the placenta. Both fetal and maternal injections were incomplete; the vinyl acetate did not penetrate into the finer channels. 305. 2 X.

- Fig. 1. Latex cast of maternal arterial (light) and venous (dark) blood vessels in the placenta, viewed from the maternal aspect. 281-3R. 3 X.
- Fig. 2. Latex cast of maternal venous blood vessel of the placenta as seen from the maternal aspect. The large venous channel (arrow) passes through the placental pedicle and eventually joins the uterine vein. 295-1. 3 X.
- Fig. 3. The same latex cast seen in Fig. 2, viewed from the fetal aspect. The fetal arterial vessels have been partially injected (darker vessels). 295-1. 3 X.
- Fig. 4. A major maternal arterial channel entering the placental disc from the mesoplacentarium (to the right). This is a late gestation placenta and fibrous tissue has invaded the maternal vessel. 317-R. Masson's trichrome stain. 13 X.

- Fig. 1. Cross section through the umbilical cord several millimeters from the body wall of the fetus. The three large allantoic vessels are identified in the upper part of the section. The two smaller, more darkly staining blood vessels in the lower portion of the section are the vitelline artery and vein. The remnant of the endodermal vesicle of the yolk sac may be seen slightly below the vitelline blood vessels (arrow). 303-2. Masson's trichrome stain. 40 X.
- Fig. 2. Cross section through the umbilical cord just beyond the level of the junction with the vitelline stalk. The allantoic blood vessels of the cord are filled with India ink. The vitelline blood vessels are within the vitelline stalk (upper part of section) which has nearly separated from the cord. Arrow indicates the allantoic endodermal vesicle. 297. Masson's trichrome stain. 13 X.
- Fig. 3. Latex cast of maternal (dark) and fetal (light) blood vessels in the placenta as seen from the fetal aspect. 281-3R. 3 X.
- Fig. 4. Latex cast of the fetal venous blood vessels in the placenta viewed from the fetal aspect. 49-A. 3 X.

- Fig. 1. Longitudinal section of a placental lobe. The black areas are fetal vessels filled with India ink. 302-2. Hematoxylin and eosin. 30 X.
- Fig. 2. Cross section of a placental lobe showing the large maternal arterial channel in the central zone. The black areas are fetal blood vessels filled with India ink. 302-2. Hematoxylin and eosin. 40 X.
- Fig. 3. A branch of the umbilical vein penetrating a placental lobe. The umbilical cord is below the lower left margin of the picture. 302-2. Hematoxylin and eosin. 50 X.
- Fig. 4. The labyrinthine zone of a placental lobe. The black areas are fetal blood vessels filled with India ink. 302-2. Hematoxylin and eosin. 100 X.
- Fig. 5. A more highly magnified section of the labyrinthine zone shown in Fig. 4. The trophoblast tubes filled with maternal blood and the fetal capillary tubes (dark areas) may be seen in longitudinal section. 302-2. Hematoxylin and eosin. 400 X.

A series of drawings to illustrate the development of the placenta of the chinchilla from implantation to the definitive condition.

Abbreviations

AL	-	allantois
AM		amnion
AEM	-	amnicembryonic mass
		cleft in uterine stroma
CE		chorionic ectoderm (lamina)
		chorionic mesoderm
		decidual cavity
		epamniotic cavity
ETB		
EMD		
		exocoel
		implantation chamber
		inner cell mass
		labyrinthine zone
		maternal arterial channel
		mesometrium
		mesoplacentarium
MV	-	maternal venous channel
SPC	-	subplacental cavity
SYM	-	symplasma
		trophoblast
		trophoblast giant cells
		Träger
		trophospongium
		umbilical cord
		uterine lumen
		vitelline stalk
		yolk sac
10	-	JOTU DOG

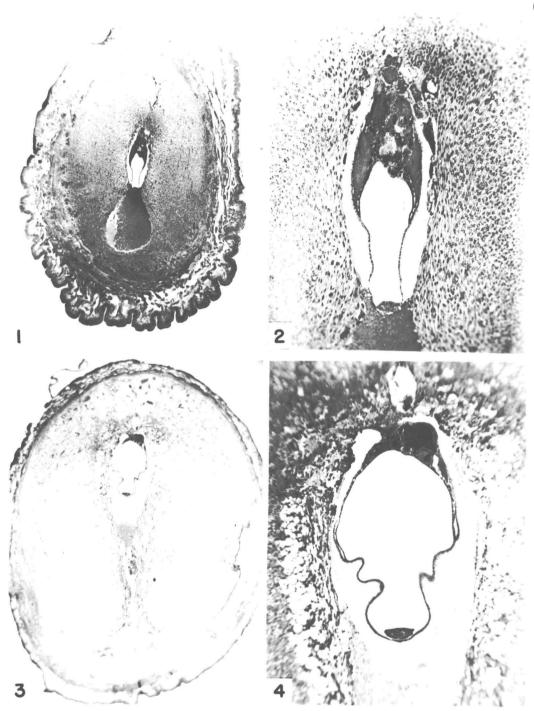
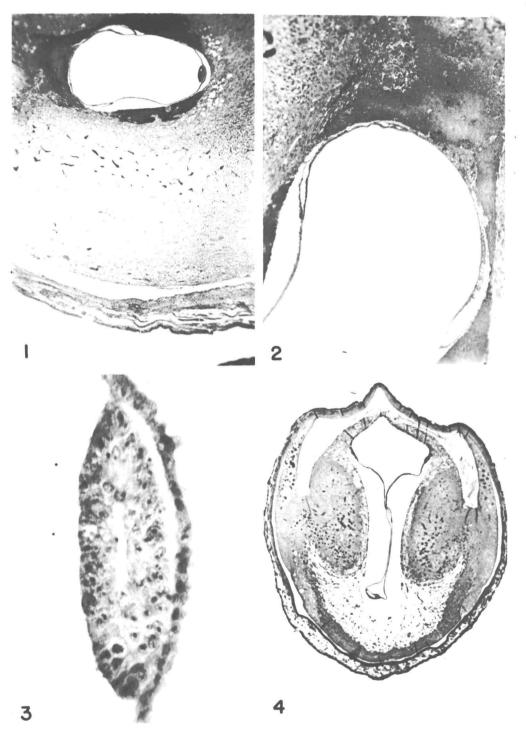
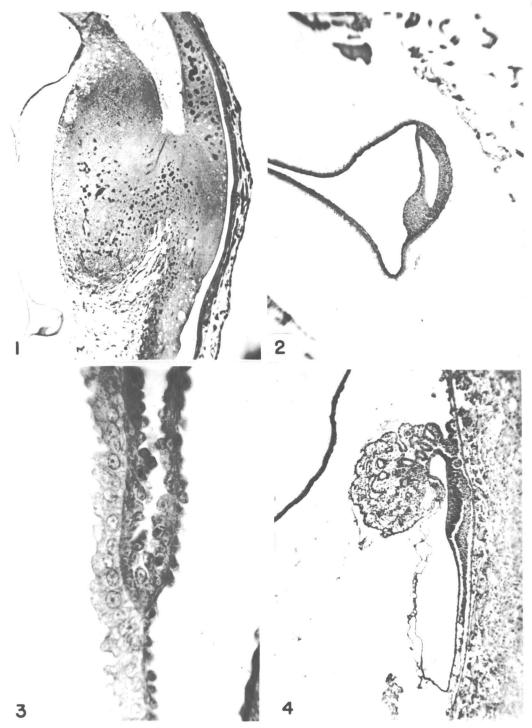


PLATE 1









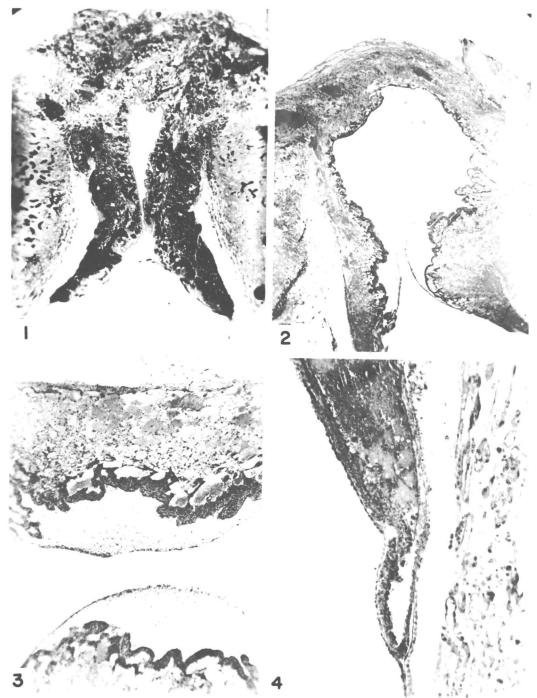


PLATE 4

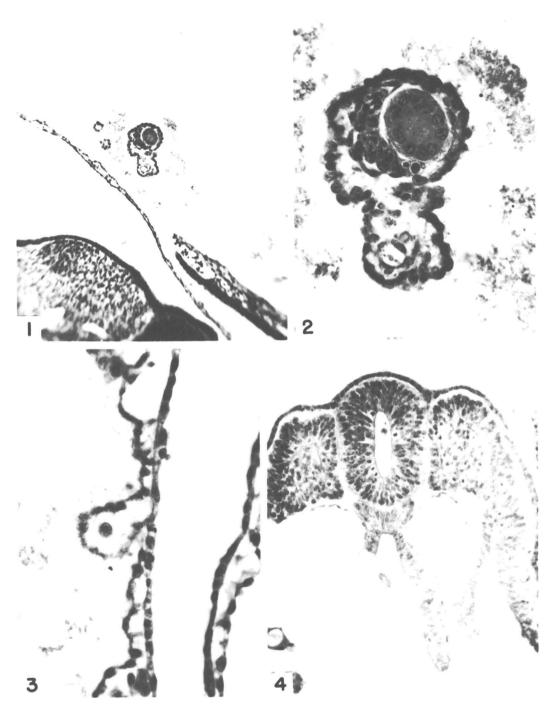


PLATE 5

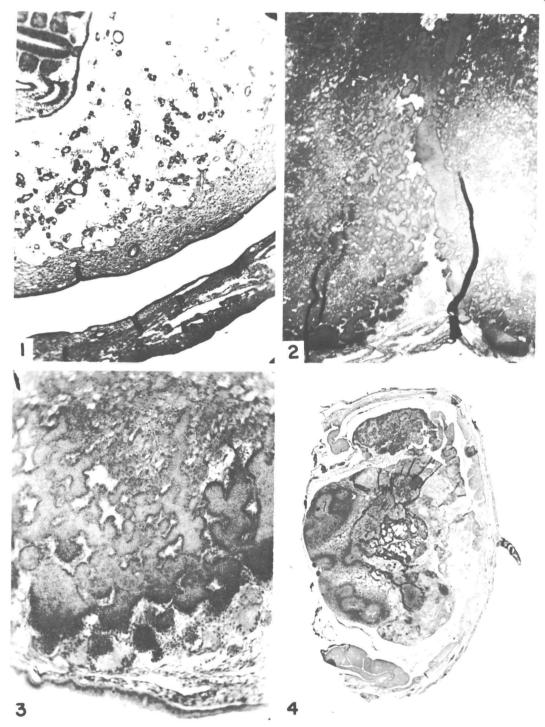
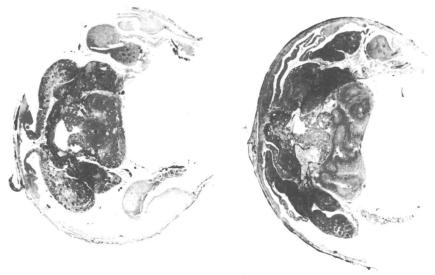


PLATE 6



I.

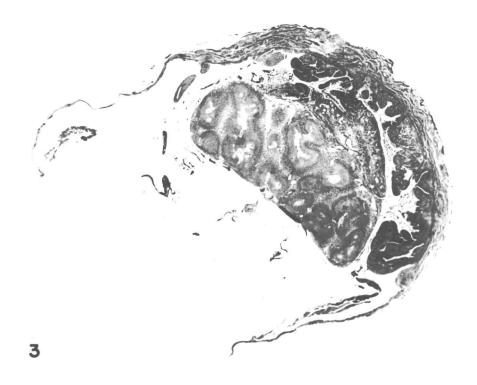


PLATE 7

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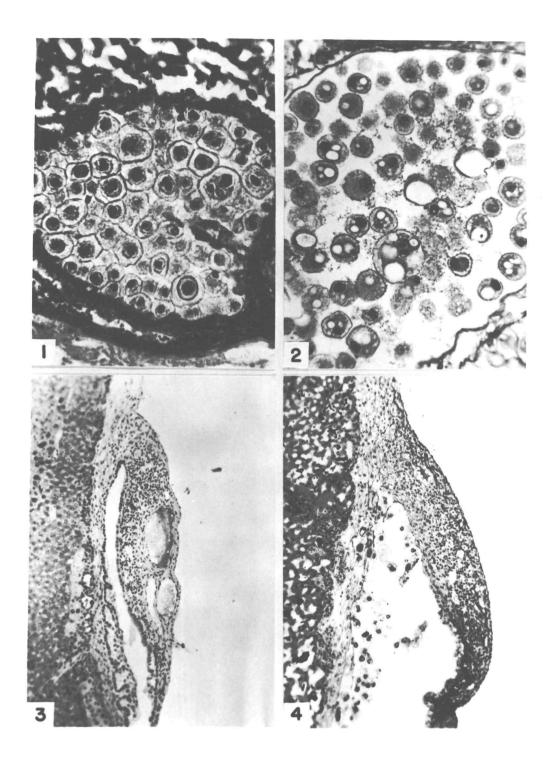


PLATE 8

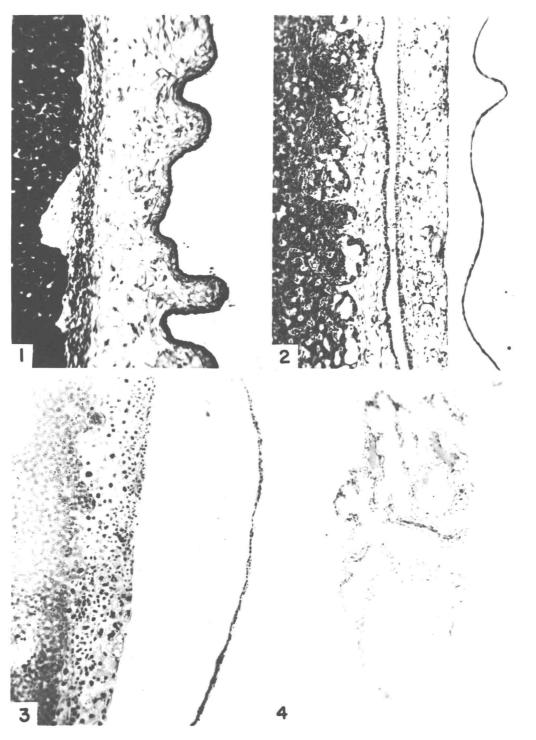


PLATE 9

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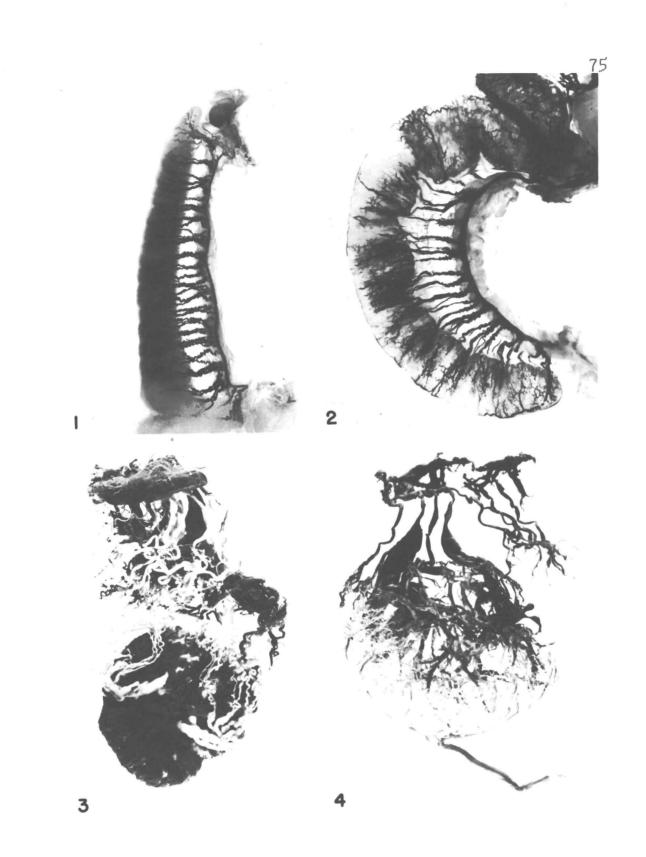


PLATE 10



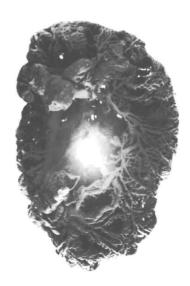






PLATE 11



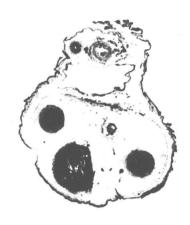
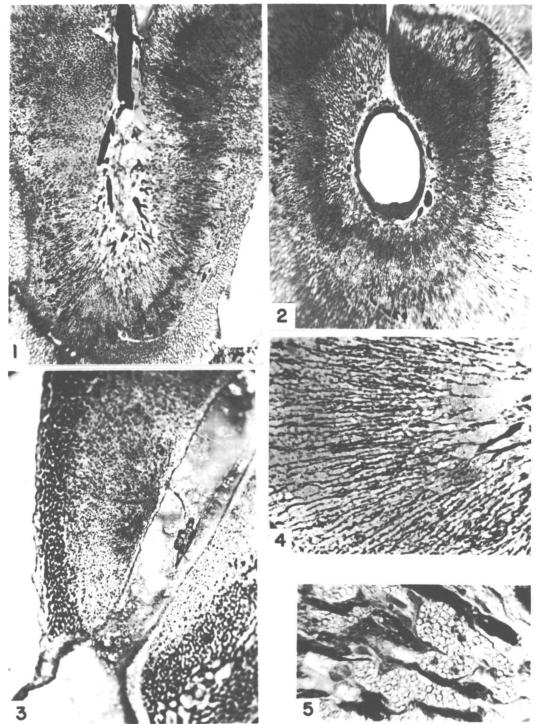


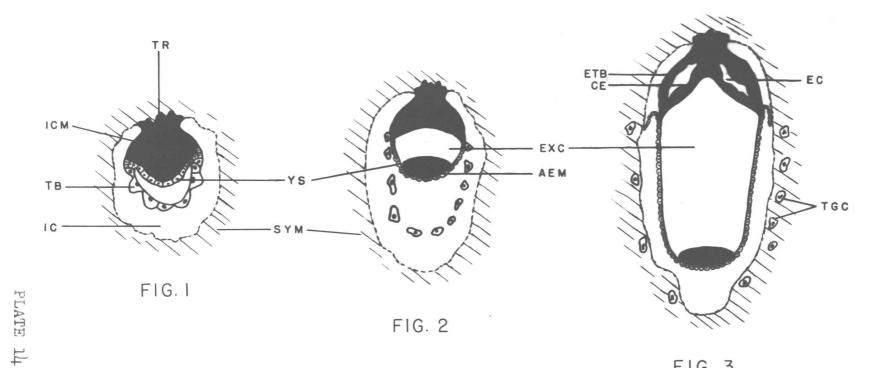




PLATE 12









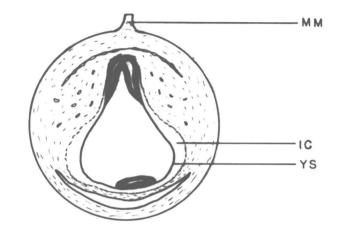


FIG. I

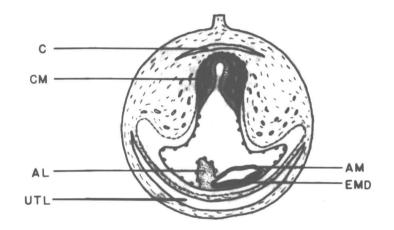
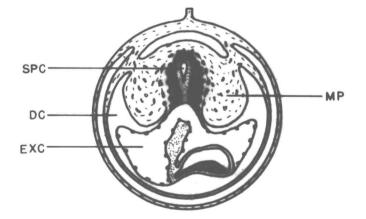
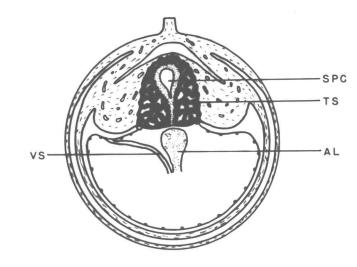


PLATE 15









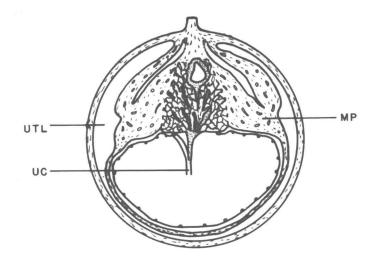


FIG. I



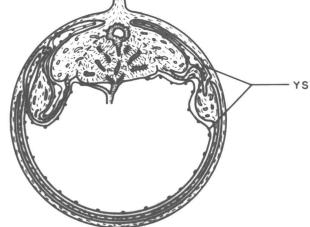


FIG. 3

PLATE 16

PLATE 17

FETAL BLOOD VESSELS NOT SHOWN

