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THE FETAL MEMBRANES AND PLACENTATION OF THE TROPICAL AMERICAN VAMPIRE BAT DESMODUS ROTUNDUS MURINUS

with Notes on the Histochemistry of the Placenta[1]

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Studies of the reproduction, embryology and placentation of bats, while numerous, deal unfortunately with only about half of the 17 families of Chiroptera currently recognized (cf. *Simpson* [1945]). The general reproductive habits have been more or less adequately worked out in representatives of only 8 families (cf. *Wimsatt* and *Trapido* [1952] for review of the literature), and the details of early development and placentation are likewise known in only 8 families, the Pteropidae (cf. *Moghe* [1951] for review of the literature), Rhinopomatidae (*Srivastava* [1952]), Emballonuridae (*Gopalakrishna*, unpublished), Megadermatidae (*Gopalakrishna* [1950]), Rhinolophidae, Hipposideridae, Phyllostomatidae, Vespertilionidae and Molossidae (cf. *Wimsatt* [1944] for literature).

The present paper will record some detailed observations upon the early development and placentation of the vampire bat, *Desmodus rotundus murinus*, of the family Desmondontidae. The unique reproductive habits of this species have recently been described (*Wimsatt* and *Trapido* [1952]), but with the exception of a brief summary (*Wimsatt* [1950]), no account of the early development and

[1] This study was made possible only through the kindness of Dr. *Harold Trapido* of the Gorgas Memorial Laboratory in Panama, who devoted considerable time and effort to the collection, preservation and shipping of the vampire material. His unstinting cooperation is gratefully acknowledged. Thanks are also due to Dr. *Herbert C. Clark*, director of the Gorgas Laboratory, for permitting the use of the facilities of the laboratory, and for bearing the burden of expense incident to the procurement of the material.

placentation of any member of the Desmodontidae has yet been published. It will be shown that *Desmodus rotundus* differs markedly from most bats thus far described in several aspects of the early development of the fertilized ovum, the mode of implantation, the formation of the amnion, the character of the decidual membranes, and the structure of the yolk-sac placenta. With respect to these, the development of the vampire bat most nearly resembles that of the tropical American fruit bats of the family Phyllostomatidae, as described by *Hamlett* [1934, 1935] and *Wislocki* and *Fawcett* [1941]. The similarity is not surprising for the Desmodontidae and Phyllostomatidae appear to be closely related taxonomically, being placed by *Simpson* [1945] in the same superfamily, Phyllostomatoidea. Further, it will be shown that the labyrinthine allantoic placenta of *Desmodus rotundus* is of the endotheliochorial type, an observation that was unexpected inasmuch as the majority of bats are alleged to have hemochorial placentas, but especially because both *Hamlett* [1935] and *Wislocki* and *Fawcett* [1941] state that the placenta of the closely related Phyllostomatidae is hemochorial.

A few observations on the histochemistry of the placenta and fetal membranes in late gestation will also be presented. These include the distribution of ribose nucleic acid, glycogen, glycoproteins and alkaline phosphatase.

Material and Methods.

The bats used in this study were collected between 1945 and 1948 from the Chilibrillo caves in Panama, or from a hollow tree near Juan Mina, Canal Zone. There were available complete serial sections of seven reproductive tracts containing embryos ranging from cleaving tubal ova to fully implanted specimens of nearly 2 mm. and incomplete series of eight uteri containing fetuses ranging from 8 mm to 11 mm, the latter being near term. All of these specimens had been fixed in 10 per cent formalin or *Bouin*'s fluid, embedded in paraffin, and sectioned at 5 to 8 micra. They were variously stained, often alternately in the same series, with *Harris*'s hematoxylin and eosin, *Heidenhain*'s iron alum hematoxylin, *Mallory*'s [1938] phosphotungstic acid hematoxylin, the *Mallory* azan stain, and the *Masson* trichrome procedure.

In addition to the above, portions of the placentas and fetal membranes of specimens from the latter half of gestation were fixed and stained in a variety of ways for histochemical analysis. The fixatives include *Zenker*'s and *Maximow*'s fluids, the alcohol-formalin mixture of *Scott* [1933], *Rossman*'s alcohol-formalin-picric acid mixture, and cold 80 per cent alcohol. These materials were embedded in paraffin, sectioned at 5 micra and stained as follows: eosin and methylene blue, both with and without previous treatment with ribonuclease (for demonstration of ribose nucleic acid); the *McManus* [1946] periodic acid-*Schiff* (PAS) procedure, both with

and without previous treatment with saliva (for staining of glycogen and glycoprotein-): the *Mitchell* and *Wislocki* [1944] modification of the Pap procedure, *Gomori*'s [1937] silver oxide procedure, and *Gomori*'s [1946] methenamine silver procedure (for demonstration of reticular fibers, glycogen and "mucins"); *Weigert*'s resorcin-fuch-in (for elastin); and *Gomori*'s [1941, 1951] calcium carbonate, and diazo dye procedures for alkaline phosphatase.

General Observations and Discussion.

Gross morphology of the female reproductive tract.

The structure and relations of the uterus, oviducts, ovarian bursae, and ovaries are revealed in the accompanying sketch (fig. 1) which was drawn under the dissecting microscope. The vagina was extirpated at the level of the fornix and is not included. A study of sections of this specimen revealed that the animal was in proestrum when killed. The view is from the ventral aspect.

The uterus is bicornuate, as in the majority of chiropterans thus far investigated. The short, cylindrical cornua diverge only slightly from the axis of the relatively long corpus uteri. The cornua are symmetrically developed, a feature which distinguishes the uterus of *Desmodus rotundus* from that of many species of vespertilionid and rhinolophid bats in which the cornua are unequally developed, the right one being generally larger than the left (*Mathews* [1937], *Wimsatt* [1944]). In the latter instances pregnancy normally occurs only in

Fig. 1. Drawing of bicornuate uterus and adnexa of *Desmodus rotundus*. Description in text.

the larger horn of the uterus, but in the vampire as in other bats with symmetrical uteri, pregnancy may occur in either horn. The lumina of the cornua join in the corpus uteri. The cervix is relatively long, but is not demarcated from the corpus externally. The cervical os is conical and prominent; the undivided cervical canal opens at its apex. The round ligaments of the uterus (*lig. teres uteri*) are prominent, and are attached at the apices of the cornua just lateral to the insertion of the oviducts.

The oviducts are relatively long, and irregularly coiled. Each oviduct enters the uterus at the apex of the cornu on its anterior medial aspect, the approach being made at an acute angle from the lateral side. The lumen of the intramural portion of the oviduct is greatly constricted, but widens to enter the cavity of the cornu, anteriorly. There is no oviducal papilla. At its distal end the oviduct enters the wall of the ovarial bursa, narrows to form a short isthmus, and then opens out on the inner surface of the bursa into a number of thin fimbriae which project into the periovarial space near the attachment of the ovarian ligament (*lig. ovarii proprium*) to the ovary. Adjacent to the oviducal fimbriae the bursa is pierced by a slit-like fimbriated opening through which the periovarial space communicates with the peritoneal cavity.

Microscopic structure of the uterus and the preimplantation changes.

The histological organization of the uterus of *Desmodus rotundus* is similar to that of other bats having bicornuate uteri, but the progestational reaction which follows ovulation is more pronounced than in most other species, and is presumably related to the different mode of implantation in Desmodus. Between proestrus and implantation the volume of the uterus may be increased several fold.

In the cross-section of the immature uterus (fig. 10) the cavity appears roughly triangular in outline, the apex being directed mesometrially. A ridge-like elevation of the endometrium projects into the lumen from the antimesometrial side. The endometrium is thin, and densely cellular, and is not sharply demarcated from the muscular layers. The uterine epithelium is simple columnar and non-ciliated, and continues without morphological change into shallow cylindrical crypts, the undeveloped uterine glands, which are simple, relatively few in number, and open upon all sides of the lumen. In the pregnant animal it can be demonstrated that the myometrium consists of an outer layer of longitudinally disposed fibers, and an inner less

regular layer in which muscular lamellae are oriented in circular and oblique directions, but in the immature uterus these details cannot be sharply delineated.

During proestrus and estrus the principal changes in the uterus involve the endometrium; hypertrophy of the myometrium only begins late in the progestational reaction which follows ovulation. Throughout the estrogenic phase of the cycle, however, there is a progressive hyperplasia of the endometrial constituents, particularly of the glands and connective tissue. The endometrium as a whole thickens somewhat, but without appreciably losing its cellular density. The glands increase in length, by estrus extending to the full depth of the endometrium, and become branched. While it may be presumed that the growth of the glands is to a considerable extent a result of epithelial proliferation, in sections mitotic figures are only infrequently observed among the glandular cells. Hypertrophy of the epithelial cells, as manifested by an increase in height, is also evident during this period. In later proestrus, and in estrus, cells with achromatic cytoplasm and rounded, dark-staining nuclei appear in increasing numbers in the endometrium, and most probably represent connective tissue cells in initial stages of hypertrophy, but whether from physiological or degenerative causes, is unknown. Leucocytes are present within the connective tissue during the estrogenic phase, reaching a maximum in late proestrus, when they may be observed within the lumen of the uterus. In the single estrous specimen available, however, leucocytes are no longer in evidence. The endometrial vessels, difficult to distinguish in the immature uterus, become progressively more evident throughout the estrogenic phase, and form vascular networks about the necks of the glands and beneath the surface epithelium. Some of the changes described which occur during the estrogenic stage may be visualized by comparing fig. 10 of the immature uterus with fig. 11 of the uterus at estrus.

The progestational changes in the uterus are illustrated in fig. 12. The reaction, which ensues rapidly following ovulation and is presumably conditioned by an early functional development of the corpus luteum (*Wimsatt* and *Trapido* [1952]), involves nearly all constituents of the uterus, but is especially prominent in the epithelial and connective tissues of the endometrium. The surface epithelial cells and those of the glands continue to hypertrophy and acquire a characteristic wide apical supranuclear zone of cytoplasm containing secretion antecedents. Secretory activity is also initiated during this

period as manifested by the appearance of visible secretion products in the lumina of the glands and at the epithelial surfaces. The initiation of secretory activity is accompanied by a characteristic dilatation of the glands analagous to that which occurs in other mammals during the progestational phase of the cycle. Proliferation among the epithelial components, however appears to be minimal.

The principal change in the endometrial connective tissue during that part of the progestational reaction which precedes implantation involves an intensification of a proliferative process which was only slightly evident during the estrogenic phase of the cycle. Mitotic figures become much more numerous among the connective tissue cells, but before implantation there is no generalized decidual enlargement of these elements, although individual swollen cells similar to those which appeared during the estrogenic phase are not uncommon. In general the nuclear density within the endometrial connective tissue appears to be about the same at the end of the preimplantation period as at the first appearance of the progestational reaction, despite the fact that the uterus has meanwhile increased enormously in volume. Presumably the enlargement of the uterus is principally attributable to the hypertrophy and dilatation of the glands and an actual increase of the cells of the connective tissue, for edema is only slightly evident.

In the late preimplantation period the smooth muscle cells of the myometrium begin a process of hypertrophy which will become maximal in early placental stages. Whether proliferation also occurs in the muscle was not determined with certainty.

Development of the fertilized ovum up to implantation.

The material available includes 3 specimens of unimplanted embryos, viz., a cleaving ovum of 6 blastomeres (fig. 14), an older cleaving ovum in the solid morula stage, consisting of approximately 40 blastomeres (fig. 15), and a young, fully formed blastocyst (figs. 2, 16). It is apparent from these specimens that the initial processes of cleavage and early blastocyst formation follow the typical mammalian pattern. Segmentation of the ovum is apparently accomplished rapidly, with relatively little increase in the volume of the egg, for the young blastocyst is scarcely larger than the mature uncleaved (ovarian) ovum. The zona pelucida is still intact about the morula although the perivitelline space has been eliminated, but it has disappeared from about the young blastocyst. The formation of a blastocyst cavity is

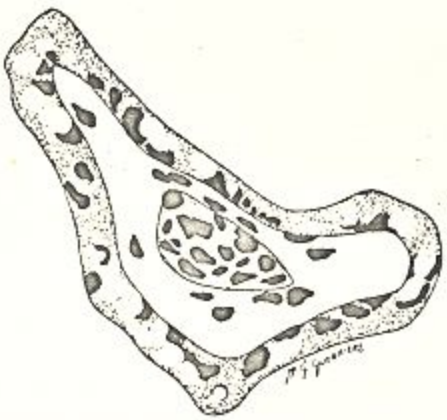


Fig. 2. Drawing of young tubal blastocyst of *Desmodus rotundus*. Precociously formed mesoblastic cells are visible just above the inner cell mass. The detached cells beneath the cell mass appear to be entodermal cells. See also fig. 16.

presaged in the morula by the occurrence of vacuoles in many of the blastomeres and of small isolated intercellular clefts between some of them. Presumptive trophoblast and inner cell mass cannot be distinguished, although this could of course be attributable to an unfavorable plane of section, and/or non-selective staining.

In the blastocyst the trophoblastic layer is everywhere unilaminar, and its cells are prominently vacuolated, but vacuoles are less evident in the cells of the inner cell mass. The cell mass lies entirely within the trophoblastic sac and comprises a solid rounded body. Beneath the inner cell mass have been delaminated a few primordial entodermal cells, and about the lateral periphery of the cell mass, and also between it and the trophoblast, are present a small number of detached cells which may possibly represent precociously formed mesodermal cells (fig. 2). This interpretation is supported by observations to be presented concerning older specimens.

An interesting feature of early development in *Desmodus rotundus* whereby it differs from most other bats that have been described is the fact that the ovum develops to the full blastocyst stage, and perhaps beyond, while still within the oviduct. The blastocyst of fig. 16, e.g. lies in the oviduct well above the uterus. Among bats a parallel condition has been described in only one other species, *Glossophaga soricina* of the Phyllostomatidae (*Hamlett* [1935]), although it is entirely probable that it occurs also in other members of this family. *Hamlett* describes and illustrates a blastocyst of *Glossophaga soricina* "just entering the uterine cavity". It consists of a fully formed

trophoblastic vesicle which contains a rounded solid cell mass. The blastocyst, which is farther along in its progress toward the uterus than that of *Desmodus rotundus*, differs from the latter, however, in two particulars, viz., there is not yet any differentiation of the cell mass into germ layers, and the trophoblast at the embryonic pole is greatly thickened and multi-layered, whereas that of the Desmodus blastocyst is not noticeably thicker at the embryonic pole than elsewhere. *Hamlett* concludes that the precocious hypertrophy of the trophoblast over the embryonic pole of *Glossophaga soricina* is in preparation for the burrowing cytolytic implantation characteristic of this species. Unfortunately I do not possess an older free blastocyst of *Desmodus rotundus* so it has not been possible to determine whether a comparable hyperplasia of the trophoblast at the embryonic pole also occurs in the vampire bat, but a specimen in initial stages of implantation (vide infra) does reveal a greatly thickened trophoblast over the zone of attachment (embryonic pole) to the endometrium, and since implantation in *Desmodus rotundus* is also of the cytolytic interstitial type, it is entirely possible that older free blastocysts might show a preimplantation trophoblastic hyperplasia comparable to that of *Glossophaga soricina*.

It probably may be inferred from the fact that the ovum develops to the blastocyst stage while still in the oviduct that its oviducal journey in both Desmodus and Glossophaga is relatively long, and that the delay is perhaps necessary in order that the endometrium have sufficient time to undergo the marked progestational reaction which seems to be characteristic of most species having an interstitial type of implantation. The possibility is not excluded, however, that the early development of the ovum may proceed at a more rapid rate in these bats than in other mammals, and that the oviducal journey is not relatively longer than in other species.

Implantation and the associated endometrial changes.

My material includes two pregnant uteri of *Desmodus rotundus* in which the ova are implanting. In one, the blastocyst is in an initial phase of attachment to the endometrium, in the other implantation has just been completed. In the latter specimen, owing probably to poor fixation, the embryonic cell mass is degenerate, but the trophoblast in contact with the uterus and the yolk sac are reasonably well preserved. These specimens clearly reveal that implantation in *Desmodus rotundus* is cytolytic and completely interstitial, a con-

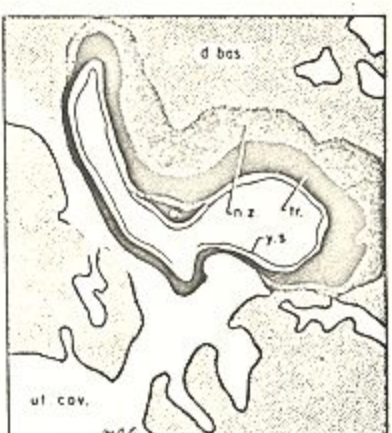


Fig. 3. Semischematic drawing of a young implanting blastocyst of *Desmodus rotundus*. See also fig. 17. d. bas., decidua basalis; n. z., necrotic zone; tr., trophoblast; ut. cav., uterine cavity; y. s., yolk sac entoderm.

dition which, as already mentioned, is found in only one other family of bats, the Phyllostomatidae (*Hamlett* [1934, 1935], *Wislocki* and *Fawcett* [1941]). The comparison is of interest because taxonomists have postulated a close relationship between the Phyllostomatidae and Desmodontidae (*Miller* [1907], *Simpson* [1945]). It might, however, be emphasized that the Phyllostomatidae possess a completely simplex uterus (*Hamlett* [1935], *Wislocki* and *Fawcett* [1941]), whereas that of *Desmodus rotundus* is bicornuate—although it is closer to the simplex form than that of any other non-phyllostomid microchiropteran thus far described[1]. *Desmodus rotundus* is the first bat possessing a bicornuate uterus in which completely interstitial implantation has been observed.

Implantation occurs at about the middle of the uterine cornu of the same side as the ovary from which the ovum originated. Attachment is effected at the antimesometrial side of the uterus by means of the embryonic pole of the blastocyst so that the orientation of the embryonic cell mass is likewise antimesometrial. The early implanting specimen shown in fig. 3, 17 is considerably larger than the small oviducal blastocyst described earlier, and has gained a firm attach-

[1] This is suggested by the relative shortness of the cornua, their less acute angle of divergence from the axis of the corpus than in other bats, the relatively greater development of the corpus uteri, and the orientation of the oviducts at their insertions into the cornua, which resemble the arrangements figured by *Hamlett* [1935] in *Glossophaga soricina*, and described by *Wislocki* and *Fawcett* [1941] in *Artibeus jamaicensis*, both of the Phyllostomatidae.

ment to the endometrium over slightly more than half of the (embryonic) surface of the vesicle; the remaining (abembryonic) surface is still exposed to the uterine cavity. Hyperplasia of that part of the trophoblast in contact with the endometrium, and cytolysis of the latter by the proliferating trophoblastic cells are the means by which attachment to the endometrium has been accomplished. The absence of uterine epithelium in contact with the implanting vesicle and the obvious cytolysis of the deeper endometrial glandular and connective tissues adjoining the thickened trophoblast indicate without question that the blastocyst plays the active part, and that implantation in *Desmodus rotundus* is entirely comparable to the process in the Phylostomatidae (*Hamlett* [1935]), the guinea pig (*Sansom* and *Hill* [1931], *Blandau* [1949]) and man (*Hertig* and *Rock* [1945]).

Within the thickened region of the trophoblast cell boundaries are indistinct and the cytoplasm is relatively achromatic. The trophoblast is still unilaminar in the free portion of the vesicle. An entodermal membrane is present and forms a continuous lining one cell thick on the inner surface of the trophoblastic vesicle, except possibly over a small area at the abembryonic pole. Beneath the embryonic cell mass the entodermal cells are somewhat hypertrophied; elsewhere they are flattened. Irregularly, in the embryonic hemisphere lateral to the inner cell mass the entoderm is separated from the trophoblast by a thin, markedly acidophilic membrane, probably an early expression of *Reichert*'s membrane, a structure which becomes quite apparent in later stages of development. The cell mass, which is not well preserved, is small and possesses a central cavity which might represent the beginning of the amniotic cavity formed by cavitation, or, merely an artifact. The latter seems most probable, for the older specimen next to be described reveals no such cavity in the degenerating cell mass, and furthermore, in *Glossophaga soricina*, in which development closely parallels that of *Desmodus rotundus*, the amniotic cavity is formed (by cavitation) much later in development (*Hamlett* [1935]).

Between the margin of the embryonic cell mass and the trophoblast, and laterally between the endoderm and trophoblast, may here and there be observed flattened dark-staining cells which probably represent precociously formed mesoderm cells. If this interpretation is correct the implanting blastocyst of Desmodus resembles that of Glossophaga in which *Hamlett* [1935] has described the origin of "primary" extra-embryonic mesoderm from the entoderm of the yolk

sac at the margin of the embryonic cell mass both before and after the formation of the primitive streak.

The second implanting specimen, in which nidation is just completed, is illustrated in fig. 4, 18. The chorionic vesicle is completely embedded in the endometrium. The trophoblast is multilayered, and thickest, over the embryonic hemisphere of the vesicle, from which strands and masses of cells penetrate into the endometrium. Over the abembryonic hemisphere the trophoblast is still, for the most part, unilaminar. Cell boundaries are indistinct in the invasive trophoblast of the embryonic pole, but the tissue does not yet constitute a syncytium. The cells are relatively achromatic, and many are vacuolated. The yolk sac constitutes a closed vesicle, which because of poor preservation, has collapsed and lies near the center of the cavity of the spherical chorionic vesicle. The entodermal cells of the embryonic hemisphere are appreciably thicker than those of the abembryonic segment. *Reichert*'s membrane (not visible at the magnifications shown) is well developed and invests most of the shrunken entodermic vesicle, except for that portion which immediately underlies the embryonic cell mass. The latter has likewise pulled away from the trophoblast and consists of a spherical mass of cells which are so degenerate that an accurate account of the actual form and relations of the cell mass at this stage cannot be given. Precociously-formed

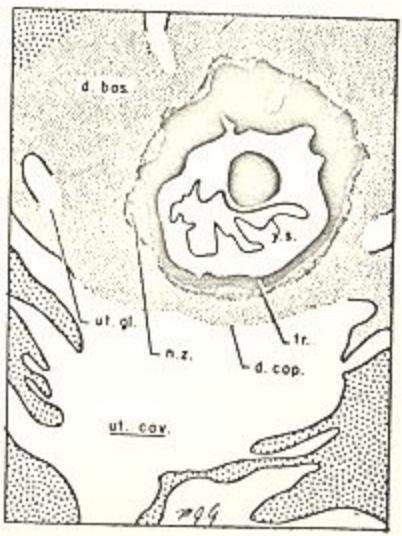


Fig. 4. Semischematic drawing of fully implanted blastocyst of *Desmodus rotundus*. See also figure 18. d. bas., decidua basalis; d. cap., decidua capsularis; n. z., necrotic zone; tr., trophoblast; ut. cav., uterine cavity; ut. gl., uterine gland; y. s., yolk sac.

mesoderm cells corresponding to the "primary" mesoderm of *Glossophaga soricina* (*Hamlett* [1935]) are represented by small dark-staining cells which for the most part remained attached to the inner surface of the trophoblast when the yolk sac shrunk away. These cells are evident only in the embryonic hemisphere of the chorionic vesicle.

In both of these implanting specimens the endometrium displays a conspicuous decidual reaction, but it is most pronounced in the older one. In each, it is restricted to those portions of the endometrium in the immediate vicinity of the implanting blastocyst, being especially prominent opposite the invasive trophoblast of the embryonic pole (figs. 3, 4). The reaction is characterized first by a marked hypertrophy, and subsequently, by vacuolation of the connective tissue cells. The vacuolated cells lie closer to the chorion than those experiencing initial hypertrophy, and at the boundary between trophoblast and decidua the vacuolated cells are obviously undergoing cytolysis. With the penetration of the ovum into the endometrium those gland ducts which lie in the path of the ovum are destroyed along with the decidual elements, and the obvious cytolytic disintegration of the glandular epithelium comprises one of the most diagnostic indications of the invasive nature of the implantation process in *Desmodus rotundus*.

Amniogenesis.

Unfortunately I possess no specimens which show the early stages in the formation of the amniotic cavity. By analogy with other bats, including *Glossophaga soricina* (cf. *Hamlett* [1935]), it is not unlikely that it had begun to form in the older implanting specimen, but the poor state of preservation of the embryonic cell mass precludes positive determination of this fact. The next oldest specimen is one in which the amnion is fully formed (fig. 20), although the embryonic disc does not yet show any evidence of primitive streak development. The fact that the amnion is already completed at this early stage of development and that the amniotic cavity contains cellular debris (fig. 20) which could only have resulted from the disintegration of more centrally-situated cells of the embryonic cell mass, leaves little doubt that the definitive amniotic cavity is precociously formed by cavitation, the floor of this cavity becoming the embryonic disc, its roof the definitive amnion. Amniogenesis in *Desmodus rotundus* thus appears to be accomplished in a comparable manner to the process in the Phyllostomatidae (*Hamlett* [1935]) and

the old world fruit bats of the family Pteropidae (cf. *Moghe* [1951] for literature). It differs from amnion formation in vespertilionid and molossid bats. In these families a primitive amniotic cavity is formed by cavitation, but the roof of this cavity, the primitive amnion, degenerates, and the definitive amnion is subsequently formed by a typical folding process (*da Costa* [1920], *Wimsatt* [1944, 1945], *Hamlett* [1934], *Mossman* [1937]). A possible exception among the latter group is provided by the vespertilionid bat *Scotophilus wroughtoni*, in which the "mode of amnion formation is similar to that described by *Hamlett* in Glossophaga" (*Gopalakrishna*, [1949 p. 239]).

The amniotic mesoderm, which is already present in the specimen described, could not have come from the primitive streak, for this structure has not yet appeared. No doubt it arises at least in part from the precociously-formed "primary" mesoderm described in younger specimens. The exact origin of the earliest primary mesoderm in the blastocyst of *Desmodus rotundus* has not been determined. *Hamlett* [1935] states that in *Glossophaga soricina* it is derived from the yolk sac entoderm where this is reflected about the embryonic cell mass, but by analogy with the human embryo the trophoblast, and perhaps the cell mass itself, are also possible sources. There can be little doubt, however, that in *Desmodus rotundus* the entoderm gives rise to primary mesoderm in later stages (vide infra). Once it has formed, the primitive streak proliferates mesoderm (the "secondary" mesoderm of *Hamlett* [1935]) which unites with the primary mesoderm so that the two cannot thenceforth be distinguished.

Yolk sac, allantois and umbilical cord.

Yolk sac: The morphogenesis of the yolk sac of *Desmodus rotundus* parallels the development of this organ in the phyllostomid bats (cf. *Hamlett* [1935] in all essential details, so the present description will be made as brief as possible. We have seen that the yolk sac entoderm begins to be formed even before the blastocyst enters the uterus[1], and that by the time implantation is in progress the entodermic vesicle is complete. With the enlargement of the embryo and the concomitant expansion of the amnion and spread of the

[1] This is somewhat earlier than it appears in *Glossophaga soricina*, for *Hamlett* [1935] describes a blastocyst of this species just entering the uterus in which the cell mass has not yet differentiated into germ layers. In all other bats for which descriptions are available the ovum enters the uterus in the solid morula stage (cf. *Wimsatt* [1944]).

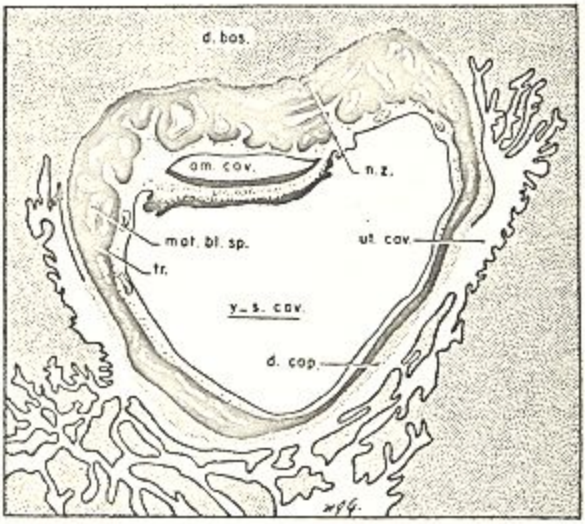


Fig. 5. Semischematic drawing of the chorionic vesicle of *Desmodus rotundus* during an early stage in the formation of the placenta, showing the relations of the fetal membranes and associated uterine structures. The thickened trophoblast (tr.) adjacent to the decidua basalis (d. bas.) marks the site of the discoidal placenta. The spaces within it (mat. bl. sp.) are maternal blood vessels which have been engulfed by the proliferating trophoblastic syncytium. The cytotrophoblast is not indicated, but may be seen at this stage in fig. 24. The thickened entodermal segment beneath the embryonic disc marks the area of entodermal proliferation of mesoblast —see also fig. 19. A primitive streak had only just begun to be organized in this specimen, so that all of the mesoblastic elements shown (small dots subtending trophoblast and embryonic disc) were precociously formed from other sources (see text). am. cav., amniotic cavity; d. cap., decidua capsularis; n. z., necrotic zone; ut. cav., uterine cavity; y-s. cav., yolk sac cavity.

exocoelom the originally spherical yolk sac is progressively infolded, its embryonic half being invaginated into the abembryonic half, so that the yolk sac cavity becomes reduced to a mere crescentic slit. Once the invagination is completed, which it is relatively early in development, the boundary between invaginated and uninvaginated segments permanently lies at, or just beneath, the margin of the discoidal placenta (figs. 5, 6, 8). This boundary also roughly demarcates the position of the maximum lateral extension of the extra-embryonic mesoderm, and accordingly the most peripheral limit of the exocoelom. Beyond this fold the entoderm of the yolk sac is

separated from the trophoblast of the abembryonic half of the chorionic vesicle only by *Reichert*'s membrane, so that there exists an extensive, permanent, bilaminar omphalopleure.

It is obvious from these relationships that only the embryonic, invaginated segment of the yolk sac, which alone is invested with mesoderm, becomes vascularized. Blood islands seem not to develop appreciably within it before the primitive streak begins to form, but once initiated the differentiation of the vitelline vascular elements is rapid, for a presomite embryo with a still-organizing primitive streak already shows an extensive development of blood cells and endothelium within the wall of the yolk sac.

Concomitantly with the vascularization of the invaginating portion of the yolk sac, the entodermal cells of this segment become markedly hypertrophied (figs. 21, 42), assuming a cuboidal or columnar form. This is in striking contrast to the entodermal cells of the avascular bilaminar omphalopleure, which not only do not hypertrophy, but become so attenuated as to be at times difficult to identify (fig. 41). Hypertrophy of the entodermal cells of the vascularized portions of the yolk sac is a generalized phenomenon in bats, and has been observed in all species for which adequate descriptions of the yolk sac are available (cf. *Wimsatt* [1944, 1945, 1949]). On the other hand, in *Desmodus rotundus*, and probably also in the Phyllostomatidae, the striking hypertrophy of the mesothelial cells facing the exocoelom in the vascularized segment of the yolk sac which occurs in the vespertilionid bat *Myotis lucifugus* (*Wimsatt* [1945, 1949]) is absent, a morphological difference between the yolk sacs of the two species which no doubt has some physiological basis.

In the angle of the yolk sac near the margin of the placenta may occasionally be seen in sections what appear to be short splanchnopleuric villi protruding into the yolk sac cavity. *Wislocki* and *Fawcett* [1941] interpret similar structures in the phyllostomid bat *Artibeus jamaicensis* as true villi, and although *Hamlett* [1935] does not mention them in *Glossophaga soricina*, villus-like structures can be seen at the same place in his fig. 34.

While *Reichert*'s membrane (figs. 41, 48) is probably not peculiar to Desmodus, its presence seems not to have been noted heretofore in any Chiropteran. It is definitely absent in the vespertilionid bat *Myotis lucifugus* (personal observation), and the fact that its presence is not mentioned in descriptions of other bats which have been

carefully studied suggests that it might generally be absent among the Chiroptera. It is somewhat surprising, in view of the close relationship of *Desmodus rotundus* and the phyllostomid bats, and their parallel course of development, that neither *Hamlett* [1935], nor *Wislocki* and *Fawcett* [1941] mention the presence of *Reichert*'s membrane in *Glossophaga soricina* and *Artibeus jamaicensis* respectively. It is evident, however, that the latter authors overlooked the structure because it may be plainly seen in their fig. 6, lying between entoderm and trophoblast. The staining characteristics of *Reichert*'s membrane in Desmodus are similar to those of the corresponding membrane of rodents (*Wislocki*, *Deane* and *Dempsey* [1946], *Wislocki* and *Padykula* [1953] and shrews (*Wislocki* and *Wimsatt* [1947]). It seems probable that the membrane has a similar chemical constitution in all of the species described.

In the preceding section it was stated that although the source of the earliest mesoderm was not determined, there can be little doubt that the entoderm gives rise to mesoderm in later stages. The process becomes evident shortly after implantation is completed, and overlaps in time the formation of the primitive streak and the proliferation by it of secondary mesoderm. The proliferation of mesoderm by the yolk sac entoderm occurs about the entire margin of the embryonic disc, with the possible exception of the area immediately behind the primitive streak, but it is most pronounced nearer the anterior end of the disc. The process resembles in every respect that described by *Hamlett* [1935] in *Glossophaga soricina* and the reader is referred to *Hamlett*'s paper for details. The proliferative zone in *Desmodus rotundus*, with its thickened entoderm and stellate mesenchyme-like cells, is illustrated in figs. 5 and 19.

In concluding this description of the yolk sac of the vampire bat it is desirable, for purposes of perspective, to compare briefly vitellogenesis in *Desmodus rotundus* with the process in other Chiroptera. Both similarities and dissimilarities are noted. In all bats thus far described the yolk sac remains large, viable and presumably functional throughout gestation. All species likewise display an "incomplete inversion", in that the embryonic half of the yolk sac is pressed by the expanding amnion into the abembryonic half, but there is no disintegration of the abembryonic omphalopleure, and hence no opportunity for contact between the vascularized invaginated segment and the uterine tissues. Hypertrophy of the entodermal cells of the vasculosa appears also to occur in all species of bats.

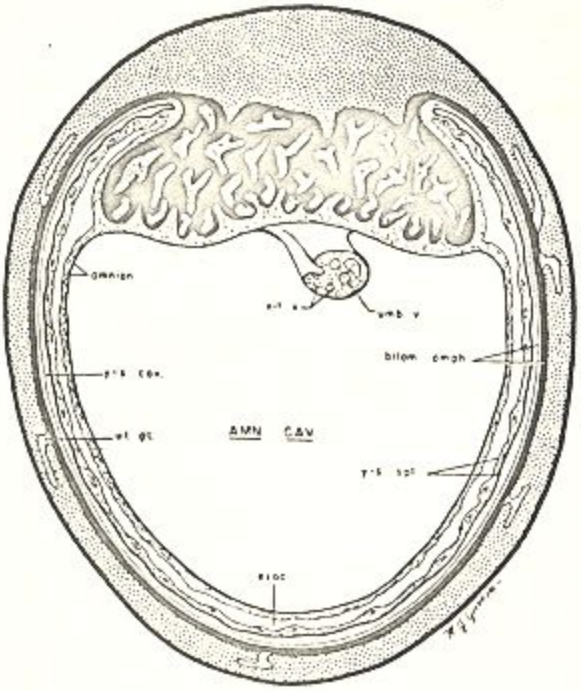


Fig. 6. Schematic representation of the definitive arrangement of the fetal membranes and placenta of *Desmodus rotundus*. Note the extensive bilaminar omphalopleure and the invagination of the roof of the yolk sac by the amnion. The narrow space between the entoderm and trophoblast of the bilaminar omphalopleure marks the site of *Reichert's* membrane (see also figs. 46, 48). The spaces labeled yolk sac cavity and exocoelom are exaggerated, and in life are only potentially present. for the amnion, vascular splanchnopleure and bilaminar omphalopleure are ordinarily in contact with one another. amn. cav., amniotic cavity; bilam. omph., bilaminar omphalopleure; exoc., exocoelom; umb. v., umbilical vessels; ut. g., uterine gland; vit. v., vitelline vessels; y-s. cav., cavity of yolk sac; y-s. spl., splanchnopleure of yolk sac.

On the other hand, with respect to such things as the extent of investment of the yolk sac by mesoderm, the relative extent of the area vasculosa, the degree to which the exocoelom invades the abembryonic half of the chorionic vesicle and frees the wall of the yolk sac (splanchnopleure) from the trophectoderm (somatopleure), and the histology of the yolk sac splanchnopleure, three distinct trends may be recognized in the Chiroptera. The first, and possibly the most

primitive, is that found in *Desmodus rotundus* and the Phyllostomatidae, in which mesoderm, area vasculosa and exocoelom extend no further than the equatorial region of the chorionic vesicle, thereby leaving an extensive, permanent, avascular bilaminar omphalopleure.

The second condition, which is illustrated by the common vespertilionid bats (cf. *Wimsatt* [1945]), is one in which the mesoderm eventually invests the yolk sac completely, converting the bilaminar omphalopleure of earlier stages into a trilaminar structure. The exocoelom extends nearly to the abembryonic pole of the vesicle, but normally does not quite complete the separation of the entire yolk sac from the chorion, so that a small trilaminar omphalopleuric segment persists until term. In this condition the area vasculosa is much more extensive than in the preceding one, and correspondingly, the area of hypertrophied entodermal cells is relatively greater. In the vespertilionid bats also, the mesothelial cells of the yolk sac splanchnopleure facing the exocoelom undergo a pronounced hypertrophy and functional specialization (cf. *Wimsatt* [1945 and 1949]) not observed in the Desmodontidae and Phyllostomatidae. The vitelline circulation does not extend into the persistent trilaminar omphalopleure, but the latter may be invaded to some extent by allantoic vessels (*Wimsatt* [1945]).

The third condition, and perhaps the most specialized from a functional standpoint, is that which obtains in the Megachiroptera—the old world fruit bats of the family Pteropidae (cf. *Moghe* [1951] for details and literature), and possibly in at least three microchiropterans, *Taphozoas longimanus* of the family Emballonuridae (*Gopalakrishna*, unpublished; cited by *Moghe* [1951]) *Tadarida cynocephala*, of the Molossidae (personal observations), and *Rhinopoma kinneari* of the Rhinopomatidae (*Srivastava* [1952]). The entodermal yolk sac becomes entirely enclosed in mesoderm, as in the Vespertilionidae, but the extension of the exocoelom eventually separates the wall of the yolk sac completely from the chorion permitting the whole yolk sac to hang free in the exocoelomic space. As might be anticipated under these conditions, vascularization of the vesicle by the vitelline vessels is complete. But the most striking characteristic of vitellogenesis in the Megachiroptera, which has been noted by several authors (cf. *Moghe* [1951] for literature), is the remarkable transformation of the collapsed, greatly folded vesicle into a compact highly vascular gland-like structure the appearance of which has led *Sprenkel* [1932] to conclude that the organ is converted into a

gland of internal secretion. The lumen is obliterated and the hypertrophied entodermal cells become rearranged into acinous groups of irregular size completely surrounded by a loose embryonic connective tissue. The mesothelial cells of the exocoelomic surface of the transformed vesicle show no structural specialization, at least in later stages (personal observation). *Robin* [1881] illustrates clearly in his fig. 57 the gross appearance of such a transformed yolk sac in Pteropus, but although *Sprenkel* [1932] and *Moghe* [1951] each present a photomicrograph of a section of the yolk sac, these are so poor as to convey no clear impression of its histological organization. Accordingly, I present in fig. 22 a photomicrograph of a section of the transformed yolk sac of a specimen of Pteropus (species unknown)[1] upon which the above brief description is based. Nothing is known at present concerning the functional significance of this unusual and interesting transformation of the yolk sac in the Megachiroptera (or the 3 microchiropterans mentioned above), and a fascinating histophysiological problem awaits solution by someone in a position to obtain sufficient fresh material.

Allantois: Although my specimens of *Desmodus rotundus* span fairly well the period of gestation I was unable to find in any of them a trace of an allantois or allantoic rudiment. This suggests that the allantois is small, non-vesicular and transient. It is probable that the formation and fate of the allantois in Desmodus parallels the history of the allantois in the phyllostomid bat *Glossophaga soricina* described by *Hamlett* [1935], which the vampire bat so closely resembles in practically all other aspects of its development. In *Glossophaga soricina* the allantois arises as a tubular diverticulum within a body stalk in an analagous way to its formation in primates, but it appears later in development and assumes a much greater relative length. According to *Hamlett* [1935] there is no evidence of an allantois in a 6-somite, 1 mm-long embryo, but it has already attained maximum relative size in an embryo of 2.5 mm. The allantois of this specimen is tubular, runs through the cord (1 mm in length) and turns laterally to extend over the face of the placenta another 0.75 mm. The placental end branches several times, but is everywhere quite narrow. The portion within the cord is reduced to a solid strand. The degeneration of the allantois is exceedingly rapid. Already, in a 2.7 mm embryo "...the extraembryonic allantoic stalk has disappeared except in the base of the cord... and as two or three short, isolated tubules in the

[1] Obtained through the courtesy of Dr. *George B. Wislocki.*

sub-placental mesenchyme" (*Hamlett* [1935], p. 344). In a 4.0 mm embryo only the umbilical portion remained, but even this had disappeared in an embryo of 6.5 mm.

It is pertinent to recall here that the allantois is known to attain scarcely more than a rudimentary development in any bat thus far described. It does appear later in development, achieve greater prominence, and persists somewhat longer in vespertilionid bats than in the Phyllostomatidae, but even in the Vespertilionidae the distal end of the rudiment expands only slightly to form a transient, flattened sac embedded in the mesenchyme beneath the fetal surface of the discoidal placenta. The initial development and definitive size of the allantois of the vespertilionid bat *Myotis lucifugus* are illustrated in figures 11 and 5 of a paper by *Wimsatt* [1945] describing the placentation of this species.

Umbilical cord: The umbilical cord of the Desmodus embryo is straight and relatively short; in a fetus at full term measuring 41 mm crown-rump it has a total length of only 21 mm. The vessels of the cord describe at most no more than one full spiral in their course through it, and the same complement of vessels is found throughout its length. This consists at term of 2 umbilical arteries, one umbilical vein, and one vitelline artery and vein, the last named being appreciably smaller in diameter than the umbilical vessels (fig. 23). This definitive complement of vessels is established relatively early in development, perhaps before mid-gestation, for it is already apparent in the cord of a 15 mm embryo. The stages available to me do not reveal the fate of the missing vitelline artery, vitelline vein and umbilical vein.

In the youngest specimen of which I possess sections of the cord 15 mm embryo) there is no trace of either an allantoic or a yolk stalk, so I am unable to indicate at what stage of development these structures disappear. It is presumed, however, that they are lost relatively early, for in the phyllostomid bat, *Glossophaga soricina*, which *Desmodus rotundus* otherwise resembles in its development, the entire allantoic rudiment had completely disappeared in embryos of 6.5 mm, and the yolk stalk was no longer present in an embryo of 2.5 mm (*Hamlett* [1935]).

Yolk sac placentation.

Mossman [1937] has distinguished three types of yolk sac placentas in mammals: a) the non-vascular yolk sac placenta, characterized

morphologically by an apposition or fusion between the bilaminar omphalopleure and the endometrium; b) the vascular chorio-vitelline placenta, consisting of apposed vascularized trilaminar omphalopleure and endometrium; and c) the inverted yolk sac placenta which is further subdivided into two types—complete, and incomplete: the first consists of the vascular embryonic segment of the yolk sac inverted against the uterine tissues—a condition made possible by the disappearance of the intervening abembryonic omphalopleuric segment; the second consists of the vascularized embryonic segment of the yolk sac inverted against the persistent bilaminar omphalopleure, which is in turn apposed to, or fused with, the uterine tissues. In certain higher rodents there is a tendency for these several types of yolk sac placentas to succeed one another in ontogenetic development, the complete type of inverted yolk sac placenta representing the definitive condition. In other mammals the incomplete type of inverted yolk sac placenta may represent the definitive type[1].

Some form of yolk sac placentation is characteristic of all bats thus far investigated. In the Megachiroptera (*Pteropus giganteus*, *Moghe* [1951]) it is a transient structure, and is obliterated about mid-gestation by the extension of the exocoelom which completely separates the vascularized yolk sac wall (splanchnopleure) from the chorion (somatopleure). Thus the yolk sac placenta of Pteropus consists first of an extensive bilaminar omphalopleure apposed to the endometrium (non-vascular yolk sac placenta), and subsequently, in consequence of the spread of the extra-embryonic mesoderm and

[1] The classification of yolk-sac placentas proposed by *Mossman* [1937] has gained universal acceptance because it represents a convenient and abbreviated means of indicating specific interrelationships between the yolk sac, chorion and endometrium. It is based, however, entirely on morphological criteria, and only at this level can it be defended at present, for it has no demonstrated physiological value. There can be no question that in many mammals the yolk sac placenta is of great functional importance during part, or all of pregnancy, but it is also true that a single morphological type of yolk sac placenta may vary in degree, time and quality of function from one mammal to another, or from one stage of gestation to the next, differences which the classification in general use does not reflect. From the standpoint of physiology, the yolk sac placenta has received less attention and is much less well understood than the allantoic placenta. It is probable, however, that the significant findings of *Brambell* and his colleagues (cf. *Brambell, Hemmings* and *Henderson* [1951]) which are based upon an experimental study of the inverted yolk sac placenta of the rabbit, and reveal the selective involvement of this organ in the immunization reactions of the fetus, will stimulate much-needed studies of the comparative physiology of the yolk sac placenta.

area vasculosa, a chorio-vitelline placenta. The latter is shortlived, however, and probably attains no great functional importance.

Among the Microchiroptera an analagous condition is observed in the Molossidae, except that here a bilaminar omphalopleure is present only during the early post-implantation period (*Hamlett* [1934], *Mossman* [1937], and the choriovitelline placenta becomes highly developed. In *Tadarida cynocephala*, for example, it is characterized by villi up to the limb-bud stage (*Mossman* [1937]). Even before mid-term, however, the spread of the exocoelom severs all connections between the yolk-sac wall and the chorion, and there is therefore no yolk sac placenta during the latter half of gestation.

In the Vespertilionidae, the most widely investigated family of the Microchiroptera, the yolk sac placenta consists successively of a non-vascular yolk sac placenta, a very temporary and relatively inconspicuous chorio-vitelline placenta, and a small (in area), permanent, non-vascular trilaminar omphalopleure of doubtful placental significance (*Wimsatt* [1945], *Gopalakrishna* [1950a]). *Mossman* [1937] has claimed that vespertilionid bats possess an incomplete type of inverted yolk sac placenta. *Wimsatt* [1945] questions this view on the grounds that if we adhere to the usual definition (that of *Mossman* [1937]), a placental relationship does not exist in instances where the invaginated vascularized roof of the yolk-sac does not either fuse with the persistent abembryonic omphalopleure or come directly in contact with the endometrium through disappearance of the intervening portion of the omphalopleure. Vespertilionid bats have an invaginated yolk sac roof, but since this does not fuse with the persistent omphalopleure, they have no inverted yolk sac placenta. In another family of Microchiroptera, the Megadermatidae, yolk sac placentation appears to resemble that of the Vespertilionidae (*Gopalakrishna* [1950b]).

Although neither *Hamlett* [1935], nor *Wislocki* and *Faweett* [1941] mention yolk sac placentation as such in the two species of phyllostomid bats which they investigated, it is quite apparent from their illustrations that conditions in the Phyllostomatidae closely parallel those now to be described in the vampire bat, *Desmodus rotundus*. In these closely related families (Phyllostomatidae and Desmodontidae) the yolk sac placentation is in one sense more primitive, and at the same time more permanent, than in any other bat yet described. It is more primitive in that the extra-embryonic mesoderm never completely invests the yolk sac—indeed never advances beyond the margin of the placental disc. (cf. p. 298 and figs. 6, 21)—with the result that there

exists an extensive, permanent, non-vascular bilaminar omphalopleure which is tightly fused at first with the overlying decidua capsularis, and later, after the disappearance of the capsularis, with the parietal decidua. For most of the gestation period, therefore, *Desmodus rotundus* (and the phyllostomid bats) possesses an extensive, functional, non-vascular yolk sac placenta. In the later phases of gestation the expansion of the amnion passively presses the invaginated yolk sac roof (vascularized splanchnopleure) tightly against the bilaminar omphalopleure over most of its surface (fig. 47), so that the former becomes for all practical purposes "fused" with the latter, and an "incomplete" type of inverted yolk-sac placenta may be said to exist. A chorio-vitelline placenta never is formed in *Desmodus rotundus*, nor in the phyllostomid bats, for the extra-embryonic mesoderm never extends beyond the margin of the allantoic placental disc, and there is, therefore, no vascular trilaminar omphalopleure, which is, by definition, the fetal component of the choriovitelline placenta.

It is thus seen, in summary, that yolk sac placentation in *Desmodus rotundus* (and phyllostomid bats) reveals three distinguishing features in comparison with other bats: a) the yolk sac placenta is permanent, and through most of gestation is of the non-vascular type; b) an incomplete type of inverted yolk sac placenta, as morphologically defined by *Mossman* [1937] is superveniently established in late gestation; and c) there is no chorio-vitelline placenta.

Concerning the physiology of the yolk sac placenta of *Desmodus rotundus* very little can be deduced merely on the basis of histological examination. That it plays a role in the progressive histolytic destruction of most of the parietal endometrium seems obvious, and it may probably be assumed that the non-vascular omphalopleure has an absorptive function, materials being passed through the omphalopleure into the yolk sac cavity, whence they might be absorbed by the hypertrophied entodermal cells of the vascular splanchopleure and transported to the fetus via the vitelline circulation. In late pregnancy, when the splanchnopleure is adherent to the inner surface of the omphalopleure, the route of transfer would be the same, except absorbed materials would not traverse a yolk sac cavity. The extent to which such transfer actually occurs, and if it does, the nature of the substances involved, are at present unknown.

The possibility also exists, albeit supported only by indirect and inconclusive evidence, that in bats the hypertrophied entodermal cells of the vascularized yolk sac splanchnopleure may have in addition

a secretory function, which would involve the elaboration of a substance that might be conveyed to the fetus via the vitelline circulation. *Wimsatt* [1948] has shown that in the hypertrophied entodermal cells of the vascular splanchnopleure of the vespertilionid bat *Myotis lucifugus* the *Golgi* element lies beneath the nucleus, adjacent to the basement membrane, rather than in the apical cytoplasm facing the yolk sac cavity. This is in general contrary to the position of the *Golgi* element in other absorptive epithelia (e.g. the intestinal epithelium). On the other hand, in known secretory epithelia (e.g. the pancreatic acinus) the *Golgi* element lies nearest the pole of the cell at which secretory discharge occurs. To the extent that the position of the *Golgi* element bears the above constant relations to the functional polarization of the secretory or absorptive cells, the basal position of the *Golgi* element in the entodermal cells of the yolk sac splanchnopleure of the bat could reflect a secretory function, and indicate the direction of discharge of the secretion. The existence of a secretory function is further suggested by the peculiar fate of the yolk sac in the Megachiroptera, and possibly certain Microchiroptera, in which, as already described, the yolk sac is transformed into a solid, vascular, gland-like organ to which some authors have attributed an endocrine significance.

It is not inconceivable that the hypertrophied entodermal cells of the vascular splanchnopleure of the majority of bats possess some of the physiological properties of those of the yolk sac of the Megachiroptera, including secretion, if the yolk sac of megachiropterans is indeed secretory. It must be reemphasized, however, that the proposed secretory function of the yolk sac is purely hypothetical, for there has as yet been no cytological characterization of a secretory product, nor any experimental proof that secretion actually occurs. The work of *Brambell, Hemmings* and *Henderson* [1951] which has unequivocally demonstrated the absorptive capacities of the yolk sac of at least one mammal, the rabbit, remains to my knowledge the only conclusive demonstration of the physiological role of a mammalion yolk sac placenta.

Allantoic placentation.

The definitive chorio-allantoic placenta of *Desmodus rotundus* is a fleshy discoidal structure which is attached by a broad, shallow pedicle of trophoblastic and decidual tissue to the antimesometrial wall of the uterus. Its rounded margin is undercut to some extent by

a fold of the bilaminar omphalopleure and the apposed invaginated splanchnopleuric segment of the yolk sac, the boundary between yolk sac somatopleure and splanchnopleure lying within the periplacental recess (figs. 6, 8). It is evident from these relationships that the disc constitutes the only segment of the chorion which is vascularized by allantoic vessels, and that accessory allantoic placental structures are absent. In latter respect *Desmodus rotundus* differs from most other bats (e.g. the vespertilionid bat *Myotis lucifugus*) in which a greater peripheral extension of the extra-embryonic mesoderm and exocoelom results in the elimination of the bilaminar omphalopleure and the establishment of a somatopleuric "membranous chorion" which is subsequently vascularized by allantoic vessels (*Wimsatt* [1945]).

Morphogenesis of the allantoic placenta. In its fundamental aspects the development of the placental disc in *Desmodus rotundus* is similar to that of the vespertilionid bat *Myotis lucifugus* (*Wimsatt* [1945], although there are differences in detail. As in Myotis, four major overlapping stages of development may be visualized. The principal developments of the first three stages are completed rapidly in early gestation and are semi-schematically represented in fig. 7, while those of the fourth stage (fig. 8) are spread over the remainder of gestation. The stages are as follows: I. The initial stage begins with implantation and is completed shortly thereafter. It is characterized by the differentiation of a sheath of trophoblastic syncytium about the embryonic hemisphere of the blastocyst. The growth of the syncytial layer is accompanied by marked histolytic erosion of the juxtaposed decidual tissue, and the progressive envelopment of endometrial vessels as the syncytial layer thickens (fig. 7, stage I). While the schematic representation of stage I depicts accurately the situation in *Myotis lucifugus* at this stage, in *Desmodus rotundus* the initiation of stage II actually occurs before the syncytial layer attains the relative proportions shown in the figure, a precocious-ness which is no doubt related to the more rapid and completely interstial implantation of the ovum in the vampire bat.

II. The second stage is characterized by the ingrowth into the syncytial mantle of solid, cytotrophoblastic cell columns which originate as villus-like outgrowths of the basal cytotrophoblast (fig. 7, stage II). These columns, of irregular thickness, are at first roughly parallel, but in consequence of irregular proliferation and branching quickly develop anastomoses, and ultimately form a three-dimensional

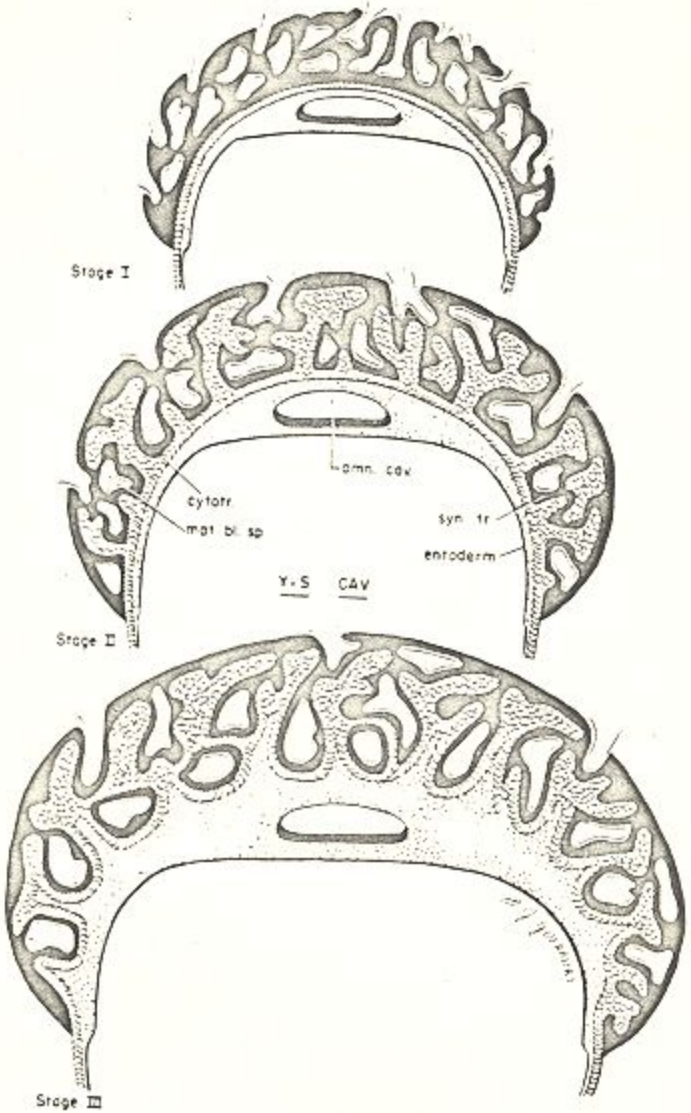


Fig. 7. Semi-chematic repre-entation of the earlier stages in the development of the di-coidal allantoic placenta of *Desmodus rotundus*. De-cription in text. amn. cav., amniotic cavity; cytotr., cytotrophoblast; mat. bl. -p., maternal blood channel within dis-c; syn. tr., syncytiotrophoblast; y-s. cav., cavity of yolk sac.

system of interconnected cytotrophoblastic cords within the syncytial trophoblast. The terminal ends of the columns form bulbous enlargements which tend to coalesce with those of adjacent cords and give rise to an incomplete plate-like sheet of cytotrophoblastic cells situated just within the maternal face of the syncytial layer (fig. 25). The cytotrophoblastic cells are thus separated from the decidua basalis only by a very tenuous sheet of syncytial trophoblast which, however, disappears in later stages permitting the cytotrophoblastic shell to come into direct contact with the decidua (figs. 8, 26). In these respects development of the placenta in *Desmodus rotundus* differs from that in the vespertilionid bat *Myotis lucifugus* in which the terminal ends of the cytotrophoblastic cell columns do not coalesce to form a cytotrophoblastic plate at the decidual face of the placenta.

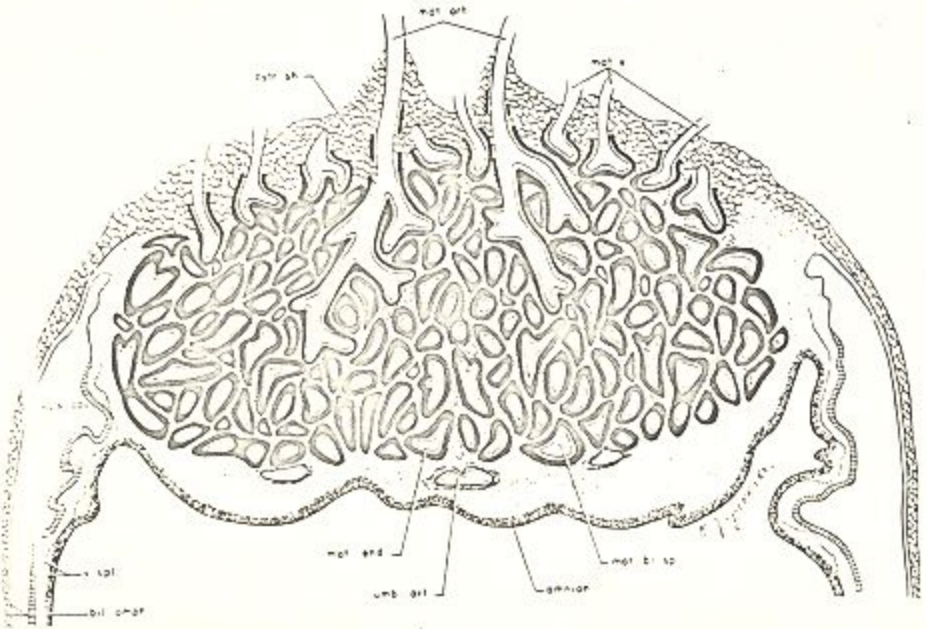


Fig. Semi-schematic representation of the structure of the definitive allantoic placental disc of *Desmodus rotundus*. Description in text. bil. omph., bilaminar omphalopleure; cytr. sh., cytotrophoblastic shell; mat. art., maternal artery; mat. bl. sp., maternal blood channel; mat. end., maternal endothelium; umb. art., umbilical artery; v. spl., vascular splanchnopleure; y-s. cav., yolk sac cavity. Syncytial trophoblast is represented by solid black, fetal mesenchymal elements by stipple.

III. In the third stage of placental development, beginning at the fetal side of the disc and proceeding peripherally, the solid cytotrophoblastic cords are "hollowed out" by the invasion into them of mesenchymal tissue, and soon thereafter, branches of the allantoic vessels (fig. 7, stage III, 24). This infiltration does not, however, extend as far as the outer cytotrophoblastic shell, which retains its solid cellular composition throughout pregnancy.

IV. The fourth stage is characterized by proliferation of all fetal constituents, the loss of most cytotrophoblastic elements except those of the peripheral shell, and a concomitant extensive internal histological rearrangement. These phenomena, collectively, provide for a vast increase of functional placental surface and the establishment of the definitive internal histological organization of the disc. The disappearance of the cytotrophoblastic cells surrounding the syncytiotrophoblastic tubules (fig. 8) is probably attributable, as in other mammals, to their transformation into syncytial trophoblast, for the latter tissue increases enormously during the enlargement of the disc, and evidence of degeneration of cytotrophoblastic cells was not observed. As a further consequence of its growth and internal reorganization during stage IV the placenta changes in gross form from its initial shape of a deep cupule to that of the definitive thick, flattened disc.

General histological organization of the definitive placenta.

The placenta in its definitive condition presents a truly labyrinthine organization, comparable to, but more complex than the placenta of the vespertilionid bat *Myotis lucifugus*, and conspicuously different from the morphologically simpler labyrinthine placentas of such species as the cat and dog in which the fetal trophoblast and mesenchymal tissues are characteristically arranged in an alternating "lamellar" pattern. In *Desmodus rotundus* the placenta consists of a complicated anastomosing system of highly branched, remarkably contorted, and aneurismic tubules of syncytial trophoblast within which are contained the material blood spaces, and surrounding the tubules a no less complicated continuous system of fetal mesenchymal tissue and branching allantoic capillaries (figs. 8, 9, 36). The syncytial tubules comprise the greater bulk of the placental tissue, the mesenchymal elements and fetal vessels being compressed into the narrow spaces between them (fig. 9). In earlier developmental stages the blood passages within the syncytium are appreciably larger in diameter on the average, and are far less numerous and contorted, than in the definitive stage.

The general histological characteristics of the fetal syncytium are similar to those of other bats and will not be described. The vascular mesenchymal tissue investing the syncytial tubules likewise presents no peculiarities or unusual cell forms. The mesenchymal fibrils are for the most part of small caliber, and in my hands were readily impregnated by *Gomori's* [1937] silver oxide methode for reticulum, and much less satisfactorily by *Pap's* method for reticulum as modified by *Mitchell* and *Wislocki* [1944]. The fibrils are most abundantly concentrated about the syncytial tubules and the allantoic capillaries (figs. 29, 37).

The cytotrophoblastic shell. A lamina of cytotrophoblast comparable to that which in *Desmodus rotundus* separates the syncytial components of the placental disc from the decidua basalis has not to my knowledge been described in any other bat. It appears to correspond to a similar cytotrophoblastic shell in the human placenta (*Wislocki* and *Bennett* [1943]). The shell in the vampire bat is a lamina of irregular thickness, but is usually best developed nearer the central region of the pedicle which attaches the disc to the uterus. On the placental side it is continuous with the cytotrophoblastic lamina investing the syncytial tubules before this layer disappears. The shell is penetrated by the maternal vessels passing between the endometrium and the placental disc, and about them forms cuffs of cytotrophoblast which follow the vessels a short distance both into the disc and into the decidua basalis. It is apparent that the cells of the trophoblastic shell perform at least two important functions. Not only are they capable of exerting a protelytic influence upon the adjacent decidua as clearly manifested by the dissolution of decidual cells and fibers at their plane of junction (fig. 26), but they also provide for placental growth by serving as a permanent germinal bed for the elaboration of new syncytial trophoblast. Sleeve-like prolongations of syncytium enwrapping the larger penetrating maternal vessels extend into the shell substance beyond the level of the regular syncytial tubules. In addition, there appear here and there within the shell isolated masses of syncytial trophoblast, most of which lie contiguous to the endothelium of penetrating vessels (figs. 8, 27), but some of which are scattered at random among the cytotrophoblastic cells. It seems clear that after the cytotrophoblast investing the syncytial tubules within the disc has disappeared, the cells of the trophoblastic shell continue to provide a source of syncytium for the investment of newly-formed blood spaces at the decidual face of the disc as the latter continues to thicken.

The finer structure of the placental barrier. I have stated in the introduction that the allantoic placenta of *Desmodus rotundus* has an endotheliochorial structure. This is contrary to a statement I made in an earlier abstract (*Wimsatt* [1950]) that the placenta is of the hemochorial variety, and it also fails to accord with the prevailing conception of placental structure in the majority of bats, in which a hemochorial condition has been described. It is therefore necessary to present in detail the evidence upon which the present conclusion concerning the endotheliochorial nature of the barrier in *Desmodus rotundus* is based.

I can assert at the outset that the earlier misinterpretation of the structure of the barrier in the vampire bat is attributable to two things, the structural peculiarities of the endothelial membrane within the syncytial tubules, and the use of routine "oversight" staining procedures (e.g. hematoxylin and eosin) which do not discriminate clearly between the maternal endothelium and the underlying tissues of the placental barrier. Unlike the maternal endothelium in the endotheliochorial placentas of such forms as the cat (*Wislocki* and *Dempsey* [1946]) and shrew (*Wimsatt* and *Wislocki* [1937]) which becomes appreciably thickened and chromophilic, that of *Desmodus rotundus* remains thin and achromatic. Attempts to stain it selectively, including staining with acid (Orange g) and basic (toluidine blue O) dyes over a range of pH (method of *Singer* [1952]), were generally unsuccessful. Furthermore, whereas the endothelial nuclei in the cat and shrew are numerous, rounded and conspicuous, those of *Desmodus rotundus* become increasingly sparsely distributed as development proceeds, and in the definitive stages of the placenta occur only at very wide intervals so that usually many sections of tubules must be diligently searched before one is found. Their recognition is rendered the more difficult in ordinary preparations because many of them become enlarged and rounded and are easily confused with the bulging nuclei of the adjacent syncytial trophoblast. The increasing scarcity of maternal endothelial nuclei in successively older placentas in combination with the failure of the endothelial membrane to thicken suggests that the increase in placental surface is accompanied by a marked stretching of the endothelial membrane without appreciable increment of cytoplasm. In view of the above considerations it is not surprising that the endothelium was overlooked initially in *Desmodus rotundus*, and more than likely in other species of bats as I shall attempt to show elsewhere.

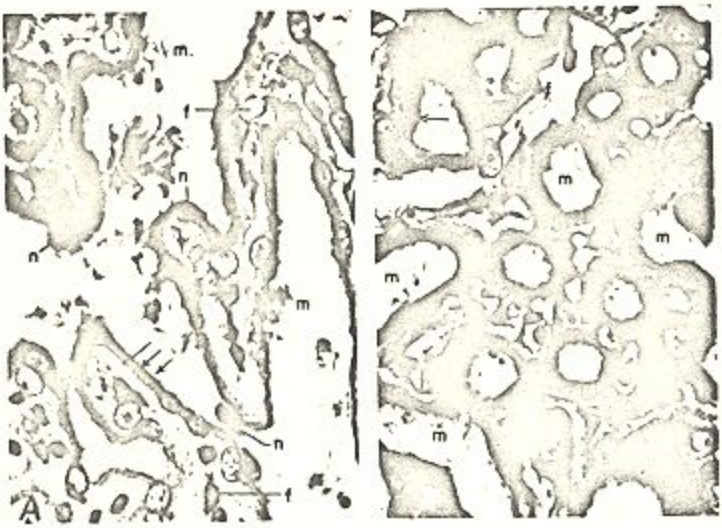


Fig. 9. Photomicrographs of the allantoic placental labyrinth of *Desmodus rotundus* near term. Both sections were stained by the *McManus-Hotchkiss* PAS procedure. Field A should be compared with fig. 36. In field A nuclei (n) of the maternal endothelium are visible, but none happen to be present in the maternal blood passageways (m) of field B. The three arrows in field A indicate, from top to bottom respectively, syncytial trophoblast, PAS-positive non-cellular membrane outside the syncytium, and the cytoplasm of the maternal endothelium which lies upon this membrane. In field B the PAS-positive membrane is indicated by an arrow, and the innermost endothelial lining is visible in most of the maternal blood spaces (m). f, fetal mesenchymal tissue. × 625.

Interestingly enough the discovery of the endothelial lining of the syncytial tubules in the vampire bat resulted initially not from the direct observation of it, but rather in consequence of the demonstration of a very thin basement membrane upon which it rests and which immediately separates it from the syncytiotrophoblast. This membrane can be stained in a highly selective manner (vide infra), and by virtue of the sharp contrast provided in such selectively stained preparations, a thin, but usually regular lamina of cytoplasm can be seen lying outside the basement membrane in most of the syncytial tubules (figs. 9a, 35, 38, 32), although it is sometimes not apparent in the smaller ones. This is the maternal endothelium, and its scarce nuclei can be readily identified when found in the special preparations be-

cause they too occur on the luminal side of the basement membrane opposite from the nuclei of the fetal syncytium (figs. 35, 38, 9a).

The basement membrane which separates the maternal endothelium from the syncytial trophoblast is variably developed in tubules of different size, but it is characteristic of all of them. It is thickest and most prominent in the larger blood vessels of the placental disc (figs. 28, 30), whence it diminishes to an exceedingly thin, but sharply delineated membrane in the smaller tubules (figs. 9a, 9b). Whether the layer in question is elaborated by the syncytial trophoblast, the maternal endothelium, or is a residuum of the original endometrial connective tissue which surrounded the maternal vessels before they were engulfed by the syncytium cannot yet be stated with certainty, but there is no doubt that it represents a distinct lamina and probably has connective tissue affinities, for its selective staining reactions resemble those of basement membranes and connective tissue structures elsewhere in the body. It displays, for example, a marked affinity for certain acid dyes, being stained bright blue by the aniline blue component of the *Mallory* azan stain, and intensely green with the light green of the *Masson* trichrome mixture. In addition it is stained a brilliant magenta by the schiff reagent as used in the *McManus-Hotchkiss* PAS procedure (figs. 9a, 28) and this reaction is saliva resistant. It is also readily impregnated with silver by *Gomori*'s [1937] silver oxide method for reticulum (figs. 29, 37) and is selectively stained by *Gomori*'s [1946] methenamine-silver procedure for glycogen and mucin (figs. 30, 38). The latter reaction is unaffected by saliva, indicating that the membrane has a mucoidal composition. The membrane is also differentially stained a greyish blue by *Weigert*'s resorcin-fuchsin (fig. 31), but the staining is less intense than that of elastic fibers within the walls of umbilical arteries in the same sections. The membrane is not consistently stained by the *Mitchell* and *Wislocki* [1944] modification of the *Pap* silver method for reticular fibers. Finally, in deparaffinized, unstained sections the membrane was occasionally observed to display, here and there, a faint birefringence.

The texture of the membrane, generally speaking, is not fibrous. In the *Mallory* azan and *Masson* preparations it has characteristically a homogeneous hyalin appearance, similar to that of *Reichert*'s membrane in the wall of the yolk-sac (which, incidentally, it resembles also in its selective staining reactions). In larger blood channels where the membrane has appreciable thickness a similar appearance is noted in the PAS and silver-impregnated sections, except that in places

where the membrane is shaved tangentially if often appears "vacuolated" (figs. 28, 30) and occasionally rather coarse striae are discernable within it. In the smaller tubules the membrane appears more "fibrous" in the PAS and silver preparations, but this may be an illusion resulting from the extreme thinness of the membrane in the smaller tubules as seen transection. Appearances in tangentially-sectioned smaller tubules suggest that the membrane may be fenestrated. The fact that the lamina in question is occasionally birefringent suggests, however, that it may contain some fibrous elements.

In conclusion, it might be mentioned that the membrane in question corresponds in position to a delicate connective tissue lamina which lies between the maternal endothelium and fetal syncytium in the placental lamellae of the cat (*Wislocki* and *Dempsey* [1946]). By calling attention in their fig. 5 to "...*residual* argyrophil fibrils and precipitate..." (italics mine) these authors imply that the membrane in the cat placenta is derived from the endometrial connective tissue. The question of its derivation in the bat will be considered elsewhere.

Vascular relationships in the allantoic placenta. The distribution of the fetal and maternal blood vessels within the placental disc of *Desmodus rotundus* appears to be similar in principle to that described in other bats (*Wimsatt* [1945], *Wislocki* and *Fawcett* [1941]) and in labyrinthine placentas in general (*Mossman* and *Weisfeldt*, [1939]). Large afferent maternal vessels penetrate the disc and pass toward its fetal surface, picking up a syncytial investment at the point where they enter the disc substance. In their course through the disc these vessels break up into smaller branches which are likewise predominantly inclined toward the fetal surface, and which, as they further subdivide and decrease in diameter, blend into the smaller system of unoriented syncytial tubules. The latter drain in turn into larger syncytium-clothed sinuses near the decidual face of the disc. From these arise a relatively large number of efferent veins that leave the disc over an appreciable portion of its decidual surface. In the later stages of gestation the disc is subdivided into irregular lobular units (cotyledons), each of which gives the impression of being supplied by a separate major branch of a maternal artery, and is perhaps drained by a separate system of veins.

The allantoic arteries penetrate the disc from its fetal aspect, and subdivide into smaller branches in a complementary fashion to the maternal arteries. The larger branches of the allantoic veins of course lie just within and upon the fetal surface of the disc. Thus the general

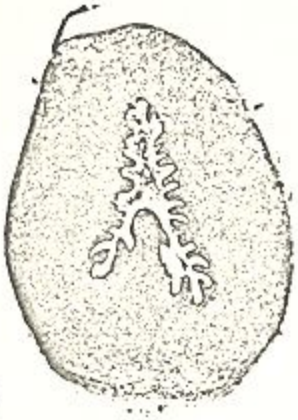
arrangement of the fetal and maternal vessels is such that the circulations within the two sets of vessels is generally in opposite directions. The physiological value of such an arrangement has been discussed by *Mossman* and *Weisfeldt* [1939] and *Barcroft* and *Barron* [1946] and will not be analyzed here.

Decidual Membranes.

Decidua capsularis. A decidua capsularis of the complete type has been described by *Hamlett* [1935] in *Glossophaga soricina*, and by *Wislocki* and *Fawcett* [1941] in *Artibeus jamaicensis*, representatives of two separate subfamilies of the Phyllostomatidae. The possibility that a *d. capsularis* is characteristic of the entire superfamily Phyllostomatoidea is strengthened by the finding in the present study that *Desmodus rotundus* possess a *d. capsularis*, at least during the earlier half of gestation. As far as known at present a *d. capsularis* occurs in no other bats.

In the vampire bat a complete *d. capsularis* is formed initially as it is in man, by a *rapid* closure of the endometrial tissue behind the burrowing blastocyst, *d. capsularis*-formation and implantation being coincident and complementary events. A feature of the *d. capsularis*

Fig. 10. The uterine horn of an immature vampire bat as seen in cross-section. The mesometrial side is toward the top of the figure. See text for description. Hematoxylin and Eosin; × 60. *Fig. 11.* Portion of cross-section of uterus of a specimen at estrus. Note greater development of glands, and sperm in glandular crypts. Magnification same as in fig. 10. Hematoxylin and eosin. *Fig. 12.* Portion of cross-section of uterus at time of implantation showing nature of progestational changes in the endometrium. Note the greater length and dilatation of the glands, the hypertrophy of the stromal cells and the thickening of the glandular and surface epithelium. Same magnification as fig. 10 and 11. Hematoxylin and eosin. – *Fig. 13.* A portion of the parietal endometrium in the region of the corpus uteri of a specimen in which the uterus contains an implanted blastocyst in an early stage of placentaformation, showing the edematous nature of the connective tissue. Description in text. Hematoxylin and eosin. × 60. *Fig. 14.* Section of cleaving ovum of six blastomeres of which only three are here visible. The ovum was situated in an upper segment of the oviduct. Hematoxylin and eosin. × 308. – *Fig. 15.* Section of a morula constituted of approximately 10 blastomeres and situated in about the middle of the oviduct. Note the still intact zona pelucida and the numerous vacuoles and clefts within and between the blastomeres. Hematoxylin and eosin. × 308. – *Fig. 16.* Section of a young blastocyst situated in the lower half of the oviduct. Note the vacuolated nature of many of the trophoblastic cells, and the small dark-stained nuclei of precociously formed mesoblast between the top of the cell mass and the trophoblast. Description in text. Hematoxylin and eosin. × 308.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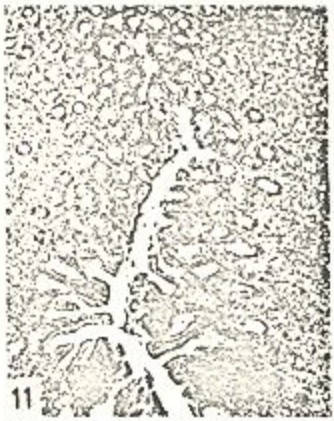
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in *Desmodus rotundus* is the absence of a covering epithelium over its exposed surface. It is probable, as a result of the rapid expansion of the implanted blastocyst and the consequent stretching of the newly-formed *d. capsularis*, that a complete epithelial covering is never even temporarily restored. My youngest specimen of the post-implantation period (figs. 5, 45) possesses a *d. capsularis* in which the naked connective tissue is exposed to the uterine lumen, and this condition is characteristic of all older specimens in which the *d. capsularis* is still recognizable. Another feature of the *d. capsularis* in *Desmodus rotundus* is the absence of a generalized decidual reaction of its connective tissue cells. Only in the proximal zone of the membrane where it adjoins the *decidua basalis* are decidually-transformed cells present in appreciable numbers.

Hamlett [1935] and *Wislocki* and *Fawcett* [1941] do not comment upon the permanence of the *d. capsularis* in *Glossophaga soricina* or *Artibeus jamaicensis*, and it is not known whether the membrane persists throughout gestation in these phyllostomid bats. With respect to *Desmodus rotundus*, an attempt has been made in the present study to determine this point. The membrane is exceedingly prominent in earlier placental stages, but as gestation advances and the chorionic vesicle continues to enlarge it becomes progressively more attenuated and less conspicuous. In a specimen in which the embryo has attained a length of 15 mm it is no longer possible to determine with certainty whether any remnants of the *d. capsularis* persist over most of the surface of the chorionic vesicle (fig. 46). At best, in the specimen illustrated, the *d. capsularis* cannot be thicker than one or two cell layers, and it seems more probable that the flattened surface cells shown are trophoblastic in origin. While there is still room for doubt

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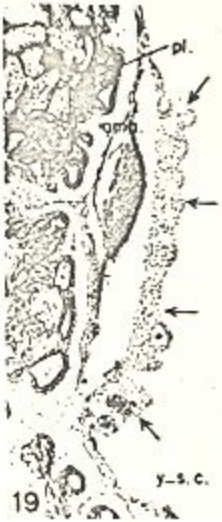
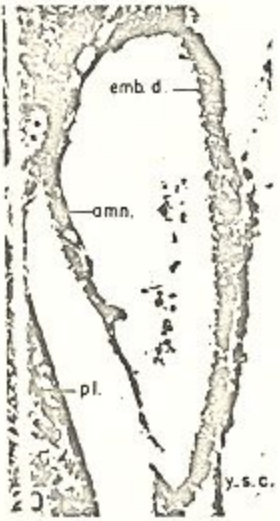
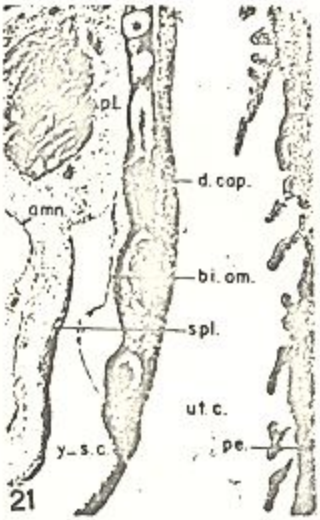
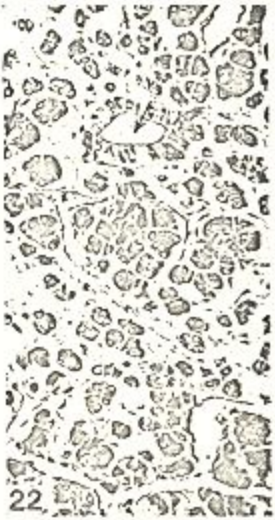
Fig. 17. Section of blastocyst in early stage of implantation. Compare with figure 3. Description in text. Hematoxylin and eosin. × 132. — *Fig. 18.* Section of fully implanted blastocyst. Compare with fig. 4. The collapsed yolk sac lies centrally. The cell mass is not included at this plane of section. Hematoxylin and eosin. × 132. — *Fig. 19.* Section through embryonic pole of chorionic vesicle of specimen shown in toto in fig. 5. The loosely thickened layer of entoderm indicated by the arrows is in the process of forming mesoblast. Opposite the lowest arrow may be seen an area in which angiogenesis has been initiated. amn., amnion; pl., placental disc; y-s. c., cavity of yolk sac. Hematoxylin and eosin. × 62. — *Fig. 20.* Section showing cellular debris within the amniotic cavity of the specimen illustrated in fig. 5 and 19. This, plus other evidence discussed in the text, suggests that formation of the definitive amnion is accomplished by cavitation. amn., amnion; emb. d., embryonic disc; pl., placenta; y-s. c., cavity of yolk sac. *Masson* stain. × 132. — *Fig. 21.* Photo-









micrograph showing relations of fetal and maternal constituents at the margin of the placenta at a somewhat later stage than the specimen of fig. 5. amn. amnion; bi. om., bilaminar omphalopleure; d. cap., decidua capsularis; p. e., parietal endometrium; pl., edge of placental disc; spl., yolk-sac splanchnopleure; ut. c., uterine cavity, y-s. c., cavity of yolk sac. Hematoxylin and eosin. × 62. — Fig. 22. Section of "solid" yolk sac of *Pteropus* (species unknown). Note arrangement of entodermal cells in separate acinar groups. Arrows indicate remnants of yolk sac cavity. *Masson* stain. × 132.

concerning the disappearance of the *d. capsularis* in the 15 mm specimen, there can be little question that the membrane has been lost over extensive areas and perhaps all of the chorionic surface of a 28 mm specimen. Details are difficult to work out in this specimen because of the firm adhesion between the fetal envelopes and the parietal wall of the uterus, and the pressure flattening of all constituents, but a characteristic relationship is illustrated in fig. 47. The flattened condition of the cells is such as were it not for *Reichert*'s membrane, which is readily identifiable between the two cellular layers of the bilaminar omphalopleure, distinction between cell layers would be virtually impossible. In the figure, immediately beneath *Reichert*'s membrane may be seen a trophoblastic layer one or two cells thick in which the nuclei are small, dark-staining and flattened. These are characteristic, and are observable over the entire surface of the flattened bilaminar omphalopleure except that portion applied to the outer wall of the periplacental groove and slightly beyond where the trophoblastic layer is unstretched and consists of large non-flattened cells. These flattened trophoblastic cells are in direct contact with endometrial, and in extensive areas, myometrial elements of the parietal wall of the uterus. In such areas as that illustrated in figure 47 there is no evidence whatever of an intermediate stratum correspond to the *d. capsularis*. Furthermore, here and there about the wall of the uterus are small areas in which the parietal endometrium possesses folds (uterine gland remnants) which still retain a covering epithelium

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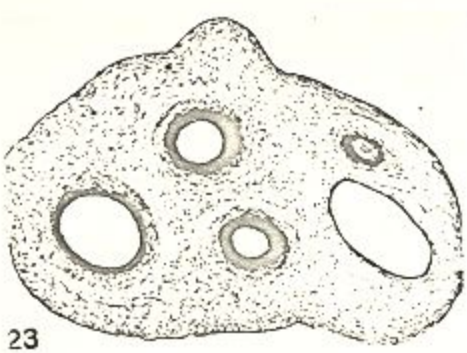
Fig. 23. Section of umbilical cord of a fetus in the second half of gestation showing definitive contents. *McManus-Hotchkiss* PAS stain. × 60. — *Fig. 24.* Section at fetal side of placental disc of specimen illustrated in fig. 5 showing penetration of fetal mesenchymal elements (m) into placental disc. amn., e., amniotic cavity; cyt., cytotrophoblast; m. l., maternal blood space; syn., syncytial trophoblast. *Masson* stain. × 308. — *Fig. 25.* Section through the central portion of the developing discoidal placenta showing an early phase of the development of the cytotrophoblastic shell. The cytotrophoblastic cells (arrows) are separated from the decidua basalis (d. bas.) by a thin, dark-staining lamina of syncytial trophoblast. *Masson* stain. × 60. — *Fig. 26.* Section showing the junction between the placental disc and the decidua basalis (d. bas.) early in the second half of gestation. The dark-staining layer crossing the middle of the figure is the cytotrophoblastic shell which at this stage is in direct contact with the basal decidua. A portion of the placental labyrinth occupies the lower third of the figure. Eosin and methylene blue. × 60. — *Fig. 27.* A maternal vessel penetrating the cytotrophoblastic shell. The arrows indicate two isolated tabs of syncytial trophoblast forming within the cytotrophoblast surrounding the vessel. cyt., cytotrophoblast. Eosin and methylene blue.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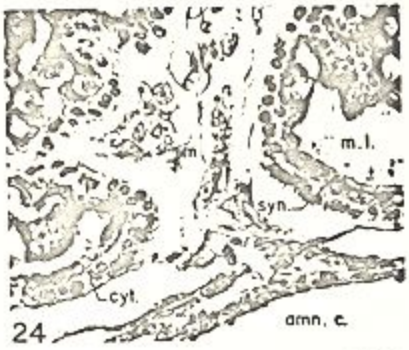

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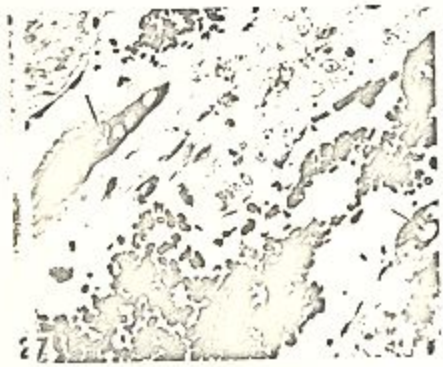
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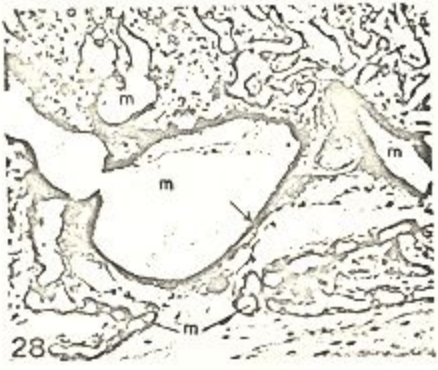

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. 308. *Fig. 28.* Placental labyrinth of the definitive allantoic placenta near term stained by the M *Manus-Hotchkiss* PAS procedure. The non-cellular membrane lying between maternal endothelium and syncytial trophoblast is heavily stained. Note its great thickness in the larger vessels and its tenuity in the smaller ones. The arrow indicates maternal endothelium. m., maternal blood spaces. Hematoxylin counter-stain. × 132.

(fig. 6). At such places the membrane consisting of the apposed embryonic envelopes (amnion, vascularized splanchnopleure and bilaminar omphalopleure respectively) has shrunk away from the uterine wall (fig. 43) and the organization of the displaced membrane may be studied independently of the constituents of the parietal endometrium. Again *Reichert's* membrane provides a useful landmark which makes it possible to ascertain that there is frequently no more than a layer or two of cells lying outside it, layers which more likely represent the trophoblast of the bilaminar omphalopleure than remnants of a *d. capsularis*.

The *decidua capsularis* of *Desmodus rotundus* is thus seen to be complete in the earlier stages of gestation, but absent in the later stages. This conclusion is a correction of a statement made in an earlier abstract (*Wimsatt* [1950]) in which it was suggested that the *d. capsularis* of the vampire bat might possibly persist in greatly compressed form throughout gestation.

Decidua basalis. In *Desmodus rotundus* the *decidua basalis* is prominent throughout most of gestation. Its formation is initiated at the time of implantation when a marked hypertrophy of the endometrial cells surrounding the embedded portions of the blastocyst first becomes prominent. The subsequent spread of the decidual reaction within the future basal endometrium keeps pace with the development and extension of the placental disc, and before mid-gestation involves the whole of the basal endometrium.

The cytological characteristics of the transformation of the decidual cells are in general similar to those described in various other mammals, the outstanding morphological change being a pronounced hypertrophy of both nucleus and cytoplasm. A special feature in *Desmodus rotundus* not observed in all mammals is the continuous formation at all stages of a relatively large number of decidual "giant cells" which very frequently possess two or more nuclei. Typically these cells appear in the necrolytic zone of the *d. basalis* adjoining the deep face of the placental disc (fig. 39). The giant cells share the fate of the other decidual cells, like them undergoing necrolysis, presumably under the influence of the nearby placental trophoblast.

In the earlier stages of placental development the decidual cells immediately adjoining the disc are destroyed in great numbers. Eventually, however, there develops adjacent to the disc what appears to be a band of specialized, vacuolated decidual cells which

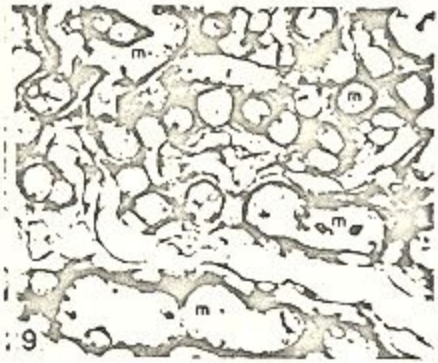
are relatively achromatic, and stand out in marked contrast to the darker staining cells of the bordering cytotrophoblastic shell and the deeper decidual layer (fig. 49). This band corresponds in appearance and position to the so-called "paraplacental layer" of the decidua described in other bats (*Wimsatt* [1948, 1949]). In *Desmodus rotundus* this paraplacental band first appears as a discontinuous lamina relatively early in placental development, and has attained maximum prominence in a specimen beyond mid-pregnancy in which the fetus measures 28 mm. This specialized strip of decidual tissue has a transient existence for it appears to be absent during the terminal stages of gestation, and indeed has already disappeared in another specimen of 27 mm. The paraplacental layer appears to be relatively more resistant to destruction by the trophoblast than the remainder of the decidua, suggesting that it acts as a temporary "buffer zone" which limits for a time the invasive activity of the trophoblast. After its disappearance (vide infra), however, direct trophoblastic erosion of the deeper decidual tissue continues.

Beneath the paraplacental layer during its existence, and, after its disappearance, adjacent to the cytotrophoblastic shell of the placental disc there develops—and becomes especially prominent during the middle third or so of gestation, a well defined zone of necrolysis containing accumulations of cellular debris and a quantity of markedly acidophilic amorphous material (figs. 26, 50). The latter appear to be products of necrolysis of bordering decidual cells of the paraplacental and deeper decidual layers, but particularly of the deeper stratum. The disappearance of the paraplacental decidua would appear to be due primarily to the degeneration of its cells facing this necrotic zone (fig. 50) rather than to direct destruction by the trophoblast. The zone of necrolysis becomes less distinct in late stages of gestation, presumably in consequence of the eventual nearly complete loss of the deeper decidual layer. Thus, in a specimen near term the cytotrophoblastic shell of the placental disc is in many places apposed to the innermost muscular laminae of the myometrium which earlier lay beneath a thick *decidua basalis*. The decidua, therefore, has been virtually eliminated at term—presumably to the nutritive benefit of the fetal tissues.

It has been suggested by *Wislocki* and *Dempsey* [1948] that a possible function of the *d. basalis* in man and other mammals in addition to the obvious nutritive one may be to protect the maternal organism by limiting the invasiveness of the placental trophoblast,

a conclusion which is based upon the supposition that the high concentration of mucopolysaccharides observed by these authors in the ground substance of the decidua would render the layer more resistant to the chemical (proteolytic) action of the trophoblast. The present observations in *Desmodus rotundus* neither support nor oppose this possibility. The nearly complete destruction of the basal decidua attests the proteolytic capabilities of the trophoblast, but mucopolysaccharides are nevertheless abundant in the decidua basalis (see p. 336) and may play a role in limiting the invasiveness of the fetal tissue. Another factor which seems not to have been previously considered is that in both Desmodus and man the trophoblastic shell consists of cyto- rather than syncytial trophoblast. There are good reasons for supposing that cellular trophoblast may have a less marked invasive potential than syncytial trophoblast.

Fig. 29. Definitive placental labyrinth of a specimen near term stained by *Gomori*'s silver oxide procedure for reticular fibers. See also fig. 37. The intensely blackened areas surrounding the maternal blood spaces (m) represent the basement membrane upon which the maternal endothelium rests. The arrow at upper left designates a maternal endothelial nucleus. Note that the blackened membrane passes beneath it, separating it from the syncytiotrophoblastic wall (light grey) of the maternal blood space. × 308. *Fig. 30.* A portion of the same placenta shown in fig. 29, but stained in this instance by *Gomori*'s methenamine silver procedure for glycogen and "mucins". The reaction shown is unaffected by salivary digestion. This figure should be compared with fig. 38 and fig. 28. Maternal blood spaces (m) are outlined by a heavily impregnated material, the same which is PAS-positive in fig. 28. This is the basement membrane which separates maternal endothelium and syncytial trophoblast. Note its greater prominence in the larger blood passageways. In the large vessel to the left the membrane has been cut tangentially and the characteristic vacuolated appearance as seen in oblique sections is clearly shown. × 308. *Fig. 31.* Same placenta as preceding, but stained by *Weigert*'s resorcinfuch-in. The positive reaction in the basement membrane (arrow) is clearly shown. Note that the staining is less intense in the wall of the larger vessel to the left in the figure. f., fetal blood vessel; m., maternal blood space. × 308. *Fig. 32.* Same placenta as preceding, but here stained in co-in and methylene blue. The intense ba-ophilia of the syncytial trophoblast is well shown. Since it is removed by ribonuclease the basophilia is attributable to ribose nucleic acid. While the maternal endothelium and underlying basement membrane are not selectively stained in this preparation both may be seen, the former resting upon the latter, opposite the arrows. m., maternal blood space; f., fetal mesenchymal elements. × 625. *Fig. 33.* General distribution of alkaline phosphatase in the placenta (right) and endometrium (left) near term. The endometrial reaction appears to be restricted to the cells of the surface and glandular epithelium. The intense reaction in the placental labyrinth is restricted to the walls of the maternal blood channels. Fixation in cold 80 % alcohol. Stained by *Gomori*'s calcium-carbonate procedure at pH 9.4, hexose diphosphate substrate, incubation time 2



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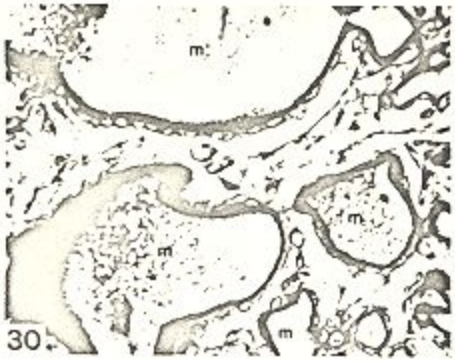

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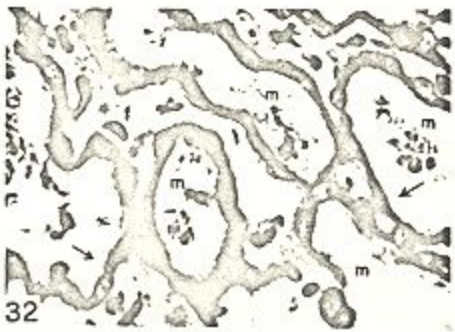

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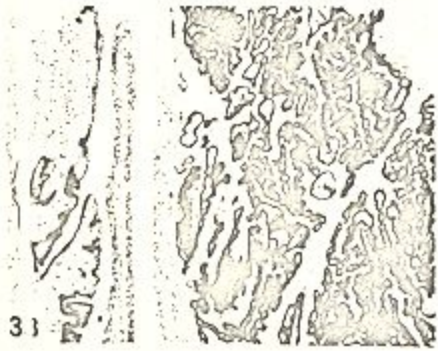

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lor rs. × 60. *Fig. 34.* Portion of placenta of fig. 33 photographed at higher magnification. Compare with fig. 36. The syncytial trophoblast displays a diffuse reaction, but the basement membrane upon which the maternal endothelium rests is intensely stained, indicating high activity. Note absence of reaction in large maternal vessel at right. m., maternal blood channel; arrow indicates a maternal endothelial nucleus. f., fetal mesenchymal tissue. × 625.

Decidua parietalis. The parietal wall of the uterus displays in *Desmodus rotundus* no generalized decidual reaction. In the earlier placental stages decidual cells are not uncommon in the proximal portion of the parietal wall of the uterus adjoining the *d. basalis*, and a few may occur elsewhere, but once the wall begins to be stretched by the expanding chorionic vesicle its endometrium gradually becomes compressed and the hypertrophied cells are no longer visible. As long as the *d. capsularis* is intact the parietal endometrium is covered by a uterine epithelium, but after the *d. capsularis* is lost the parietal endometrium is eroded away by the trophoblast of the bilaminar omphalopleure. Since the latter at term is in contact over most of its surface with the myometrial component of the parietal wall, there is scant loss of parietal tissue at parturition, so that this region is not truly deciduate. Repair of the endometrium following parturition is very rapid, for an intact endometrium and a new pregnancy have both been observed in uteri still undergoing post-partum involution (*Wimsatt* and *Trapido* [1952]).

A peculiar reaction of the *endometrium* of the corpus uteri and

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Fig. 35. Drawing of field shown in fig. 9 A, but at greater depth of focus to show better the relations of fetal syncytium, maternal endothelium and the intervening basement membrane (arrow). For further labeling and description see fig. 9 A. *McManus-Hotchkiss* PAS reaction, hematoxylin counter stain. *Fig. 36.* Drawing of placenta shown in figures 33 and 34, but at greater depth of focus to reveal the precise distribution of alkaline phosphatase activity in the placental labyrinth. The arrows indicate maternal endothelial nuclei. Note that the zone of most intense activity corresponds to the position of the PAS-positive basement membrane illustrated in the preceding figure. The syncytial trophoblast displays a less intense reaction, while the fetal mesenchymal elements (including the fetal capillary endothelium) are negative. Hexose diphosphate substrate; incubation two hours at pH 9.4. *Fig. 37.* Drawing of placental labyrinth near term stained by *Gomori*'s silver oxide method for reticulum. For description see legend of fig. 29. b., basement membrane; e., maternal endothelium; m., maternal blood channel. *Fig. 38.* Drawing of placental labyrinth near term stained by *Gomori*'s methenamine silver procedure. For description see legend of fig. 30. Arrows indicate maternal endothelial nuclei. b., basement membrane; e., maternal endothelium; m., maternal blood channel; syn., syncytial trophoblast. – *Figs. 39 and 40.* Drawing of giant cells of maternal origin in the decidua basalis near its junction with the cytotrophoblastic shell. Note the multinucleate condition of the cell at the upper right in fig. 39. Fig. 40 was drawn from a section stained by the *McManus-Hotchkiss* PAS procedure. The heavier accumulations of dark material to the right and the smaller discrete granules elsewhere represent glycogen. The paler reaction within some of the giant cells is saliva-resistant, and presumably represents glycoprotein.

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adjoining parietal endometrium of the pregnant cornu, observed in a single specimen in early pregnancy but not in another at a nearly comparable stage of development, remains to be mentioned. The embryo of this specimen is in the early primitive streak stage, and there has been an appreciable expansion of the chorionic vesicle, pushing the decidua capsularis before it. The membranes, however, have not yet expanded sufficiently to establish contact with the parietal wall of the uterus. In the regions of the endometrium mentioned above, a pronounced edema has occurred (figs. 13, 45), and the edematous portions are immensely swollen and protrude conspicuously into the uterine cavity. Edema is more pronounced nearer the endometrial surface than deeper in the endometrium. Because of it, the connective tissue cells are widely separated and the capillaries stand out sharply, and are dilated. Scattered here and there among the ordinary connective tissue cells are enlarged epithelioid cells resembling the decidual cells normally present elsewhere in the uterus. It would appear probable that the exudate responsible for the edematous condition had a watery consistency, for stained, precipitated materials are not abundant between the cells. The significance of this edematous reaction, and whether or not it represents a normal process during the earlier stages of development in *Desmodus rotundus* are unknown. I can only emphasize again that the specimen described is unique; a comparable edema has not been observed in any other specimen, including another at a corresponding stage of development.

Histochemical Observations.

The following histochemical observations were made upon placentas from the latter half of gestation, and concern the distribution of ribose nucleic acid, PAS-positive substances and alkaline phosphatase.

Ribose nucleic acid. Sections of material fixed in *Zenker's* and *Maximow's* fluids were stained in eosin and methylene blue. A few were incubated before staining in a solution of ribonuclease. Comparison of the extracted and unextracted sections revealed that the enzyme destroyed the cytoplasmic basophilia of several placental constituents. This enzyme-susceptible basophilia is presumed to indicate the presence of ribose nucleic acid.

The most intense basophilia, and presumably the greatest concentration of ribose nucleic acid, is observed in the syncytial trophoblast (fig. 32). The distribution of cytoplasmic basophilia presented the

same picture in the several late stage placentas available to me, and differs in no significant respect from that already described in another bat, *Myotis lucifugus* (*Wimsatt* [1949]). It is especially concentrated about the nuclear membranes of the trophoblastic nuclei, but is intense nevertheless in all parts of the syncytium. The basophilic material is non-granular, having the appearance of a deep homogeneous wash, even under the highest magnifications used (1350 ×). The single nucleolus of each trophoblastic nucleus is intensely stained by methylene blue, and this too is susceptible to ribonuclease treatment betraying the presence of ribose nucleic acid in the nucleoli.

The cells of the cytotrophoblastic shell, including those which form cuffs about the penetrating maternal vessels display a very pale cytoplasmic basophilia, particularly in the vicinity of the nucleus, but this is far less intense than in the fetal syncytium. Only in those cytotrophoblastic cells which are in process of hypertrophy leading to the formation of new syncytium does the cytoplasmic concentration of ribose nucleic acid approach that of the differentiated syncytium (fig. 27). As in the syncytial trophoblast, the nucleoli of the cytotrophoblastic cells are intensely stained. By contrast the adjacent decidual cells display no cytoplasmic basophilia whatever; indeed, their cytoplasm is intensely acidophilic.

Moderate cytoplasmic basophilia is also observed in the hypertrophied entodermal cells of the yolk sac splanchnopleure. The entire cytoplasm usually displays some basophilia, but the staining is most intense in the denser, apical half of the cell surrounding the nucleus (fig. 42). The basal cytoplasm beneath the nucleus contains a variable number of spherical vacuoles, which presumably contained lipid droplets, as in *Myotis lucifugus* (*Wimsatt* [1948]). The localization of ribose nucleic acid in the entodermal cells is similar to that of *Myotis lucifugus* (*Wimsatt* [1949]), but is the reverse of that reported in the yolk sac of rodents (*Wislocki, Deane* and *Dempsey* [1946]) and shrews (*Wislocki* and *Wimsatt* [1947]). In these forms the nucleus and maximum basophilia occur in the basal half of the cell. The possible physiological significance of this reversal of polarity in the entodermal cells of the bat has been discussed elsewhere (*Wimsatt* [1949]).

Periodic acid-Schiff-positive substances. Two types of PAS-positive substances are present in the placenta and adnexa of *Desmodus rotundus*. The first is removed by digestion in saliva and is presumed to be glycogen; the second is saliva-resistant and presumably consists of glycoproteins.

Glycogen is relatively restricted in distribution. None whatever was observed within the placental disc proper, or in the cells of the cytotrophoblastic shell, but appreciable deposits do occur in the walls of the umbilical vessels, especially the arteries, which ramify over the fetal surface of the *decidua basalis* (fig. 40) and in the myometrium of the uterus. The presence of glycogen in the *decidua basalis* has been reported in many mammals (cf. *Wislocki, Deane* and *Dempsey* [1946], *Wislocki* and *Wimsatt* [1947], *Wislocki* and *Dempsey* [1948], *Wimsatt* [1949]), and its presence at this site in *Desmodus rotundus* was perhaps to be expected. The possible significance of glycogen in the decidua has most recently been discussed by *Wislocki* and *Dempsey* [1948].

The only other site examined for glycogen was the visceral wall of the yolk sac. It will be recalled that the entodermal cells of the vascularized (visceral) segment of the yolk sac become hypertrophied, while those of the bilaminar omphalopleure do not. Likewise the cells of the mesenchymal epithelium on the exocoelomic surface of the visceral yolk sac hypertrophy to some extent, but in no case as prominently and characteristically as in the vespertilionid bat *Myotis lucifugus* (*Wimsatt* [1949]). Glycogen appears to be absent in the hypertrophied entodermal cells, but on the other hand it is abundant in most of the cells of the opposite mesenchymal epithelium (figs. 43, 44). It is of interest that in another bat, *Myotis lucifugus*, while glycogen is found in limited quantities in the entodermal cells, it is particularly concentrated in the mesenchymal epithelium of the yolk sac as in *Desmodus rotundus*. This distribution in bats differs

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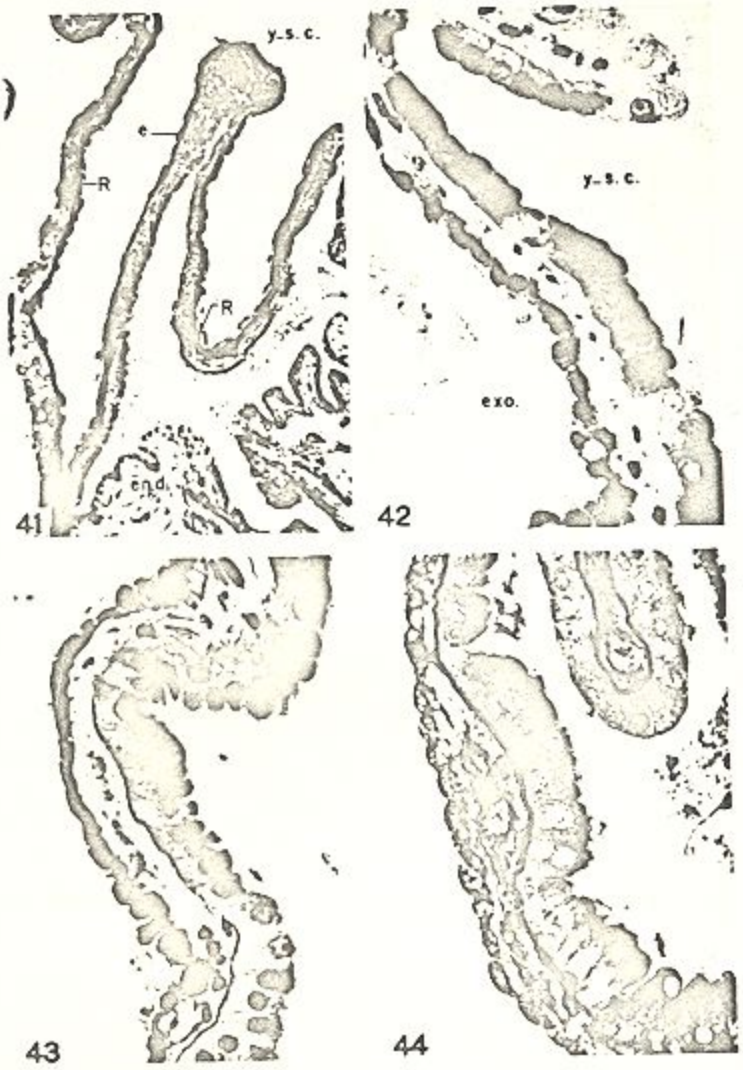
Fig. 41. Section of bilaminar omphalopleure from a specimen past mid-pregnancy in which *Reichert*'s membrane (R) has been selectively stained by the *McManus-Hotchkiss* PAS procedure. The uterus containing this specimen was fixed in toto following removal of the fetus, and the attendant collapse of the uterus produced displacement and folding of the fetal membranes. e., entoderm of bilaminar omphalopleure; end., parietal endometrium; y.s., c., cavity of yolk sac. × 132. — *Figs. 42, 43 and 44.* Sections of the vascular splanchnopleure of the yolk sac near the placental margin in late pregnancy, showing the structure and cytochemical organization of the splanchnopleure. Note in all three figures the columnar form of the entodermal cells in contrast to the irregularly flattened or cuboidal form of the opposite mesenchymal epithelium facing the exocoelom (exo.). The section shown in fig. 42 was stained in eosin and methylene blue, and the appreciable cytoplasmic basophilia of most of the entodermal cells is well shown. The sections of figs. 43 and 44 were stained by the *McManus-Hotchkiss* PAS procedure, but that of 44 was first extracted in saliva. Note the abundance of glycogen in the mesenchymal epithelium in fig. 43 and its absence from this site in fig. 44. Saliva-resistant PAS-positive granules may

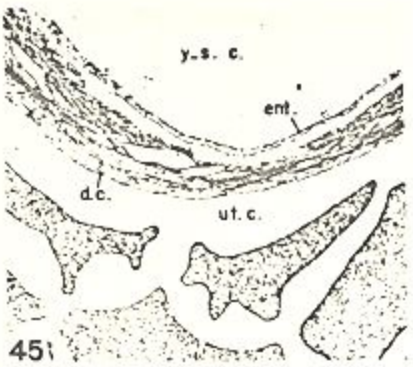


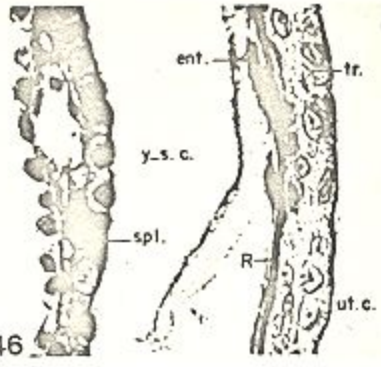
be seen in fig. 44 at the apices of most of the entodermal cells, and in many of the cells of the opposite mesothelium. The nuclei of section 43 have been counterstained with hematoxylin—they are unstained in 44. Note finally the numerous vacuoles of various sizes which lie beneath the nuclei of the entodermal cells, especially in figs. 43 and 44. Their significance is discussed in the text. × 625.

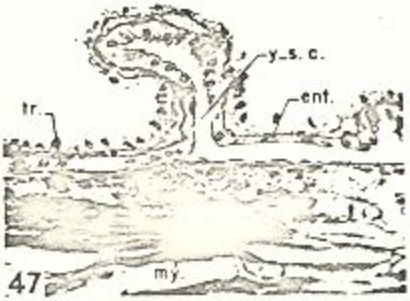
from that in some other mammals (e.g. rodents, cf. *Wislocki, Deane* and *Dempsey* [1946]. *Wislocki* and *Padykula* [1953]) in which glycogen is concentrated in largest quantities in the entodermal cells. The physiological bases for such species differences in glycogen distribution in the yolk sac are at present unknown.

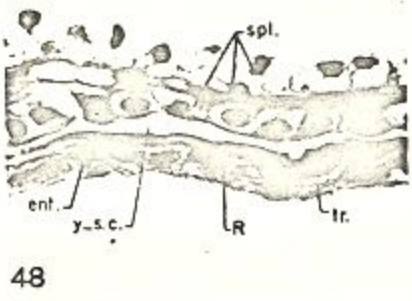
Saliva-resistant PAS-positive substances are widely distributed in the placenta, fetal membranes and uterus, and in the majority of instances are associated with connective tissue membranes and fibrils. Exceptions are the entodermal and mesodermal epithelia of the yolk sac splanchnopleure. The columnar entodermal cells show a moderate positive reaction in the apical cytoplasm surrounding the nucleus, and an occasional cell is stained uniformly pink throughout its length (fig. 44). In sections from which glycogen has been removed by saliva the moderately swollen cells of the mesenchymal epithelium facing the exocoelom are seen often to contain bright PAS-positive globules of variable number and size (fig. 44). Similar granules have been described in the yolk sac entoderm of the rat (*Wislocki* and *Padykula* [1953]). The significance of these elements has not been established.

Fig. 45. Photomicrograph of the abembryonic pole of the chorionic vesicle illustrated in toto in fig. 5 to show the histological nature of the decidua capsularis (d. c.). Note that it is thin and not covered by an epithelium. ent., entoderm of yolk sac; ut. c., cavity of uterus; y-s. c., cavity of yolk sac. *Masson* stain. × 60. — *Fig. 46.* Section of abembryonic wall of the chorionic vesicle and vascular splanchnopleure of a fetus of 15 mm. Note that there is no evidence of a decidua capsularis overlying the trophoblast of the bilaminar omphalopleure. ent., entoderm of bilaminar omphalopleure; R., *Reichert*'s membrane; spl., vascular splanchnopleure; tr., trophoblast of bilaminar omphalopleure; ut. c., cavity of uterus; y-s. c., cavity of yolk sac. Azan stain. × 565. — *Figs. 47 and 48.* Sections of the apposed bilaminar omphalopleure and vascular splanchnopleure of a 27 mm fetus. In both sections the vascular splanchnopleure is slightly separated in places from the bilaminar omphalopleure, probably as a consequence of shrinkage during fixation and removal of the fetus. The cleft like space represents, therefore, the cavity of the yolk sac. In 47 the omphalopleure is tightly fused with the parietal wall of the uterus, in places being in contact with the muscular elements of the myometrium (my.) indicating that the parietal endometrium has been destroyed. In 48 the entire wall of the chorionic vesicle has been pulled away from the parietal wall of the uterus and the structure of the bilaminar omphalopleure can be more easily visualized. ent., entoderm of bilaminar omphalopleure; m., myometrium; R., *Reichert*'s membrane; spl., vascular splanchnopleure; tr., trophoblast of bilaminar omphalopleure; y-s. c., cavity of yolk sac. Azan stain. fig. 47, × 308; fig. 48, × 625. — *Fig. 49.* Section at









boundary between placental disc and basal decidua late in the first half of gestation showing "paraplacental" decidual layer (p. d.). Several decidual giant cells may be seen within the layer (arrow). d. bas., decidua basalis; pl., placental disc. *Masson* stain. × 60. *Fig. 50.* Section showing placental disc and decidua basalis in early gestation. d. bas., decidua basalis; n. z., necrolytic zone of basalis; pl., placental disc. *Masson* stain. × 60.

Among the saliva-resistant PAS-positive constituents the most intensely stained are *Reichert's* membrane in the parietal wall of the yolk-sac (fig. 41), and the basement membrane previously described which separates the maternal endothelium and syncytial trophoblast within the placental tubules (figs. 9, 28, 35). In addition to being PAS-positive, these two membranes are identically stained by a variety of other procedures (see p. 316), and it appears probable that they have a similar composition. A thin, but distinct PAS-positive membrane underlies the entodermal epithelium of the visceral wall of the yolk-sac (fig. 44), and elsewhere in the mesenchymal tissues of the placenta and adnexa stained fibrils are numerous (figs. 9, 35). A saliva-resistant PAS reaction is also prominent in the *decidua basalis* where it is practically confined to the fibrous stroma surrounding the decidual cells. Both fibrils and an amorphous ground substance are stained. Not infrequently local concentrations of *Schiff*-positive materials occur about degenerating decidual giant cells of the outermost decidual zone, and now and then amorphous deposits are observed within the cytoplasm of such cells (fig. 40).

The significance of a saliva-resistant PAS-positive reaction has been fully discussed by other authors (*Gersh* and *Catchpole* [1949], *Leblond* [1953], *Lillie* [1952]). In general the reaction indicates the presence of polysaccharides which are presumably conjugated with protein, as in the mucopolysaccharides, some of which are PAS-positive. Evidence presented in the above reviews suggests that the intense staining of reticular fibers, and perhaps of small collagenous bundles, may depend upon the presence of a ground substance investing the fibers rather than upon the fibers themselves.

Alkaline phosphatase. The following observations are based upon a single specimen of *Desmodus rotundus* near term. Sections of tissue fixed in cold 80 per cent alcohol were stained at pH 9.4 by the *Gomori* [1941] procedure for alkaline phosphatase. Four different substrates (Na B-glycerophosphate, fructose 1–6 diphosphate, yeast nucleic acid and muscle adenylic acid) were used in the incubation mixtures. The sites of enzymatic activity were identical in all cases, but the intensity of the reactions varied with the substrate used. Nucleic acid and adenylic acid gave the most intense and least intense reactions respectively. Glycerophosphate and hexose diphosphate gave reactions of about equal intensity. In addition sections were stained by the diazo dye procedure of *Gomori* [1951], utilizing disodium-B-naphthanil phosphate as substrate and the diazonium compound Naphthanil

Diazo Blue B as a stain. The same localizations of enzyme activity were observed as with the standard calcium-carbonate procedure.

Alkaline phosphatase activity was observed in only two sites, the uterine epithelium of the parietal wall of the uterus adjacent to the placental disc (fig. 33), and the walls of the maternal blood spaces within the disc. The amnion, which gives an intense reaction in *Myotis lucifugus* (*Wimsatt* [1949]), was not examined. The yolksac splanchnopleure was completely negative in all preparations. Surprisingly, a negative reaction was also obtained in the muscle cells of the uterine myometrium, elements which in *Myotis lucifugus* are intensely reactive. No explanation is offered for this discrepancy.

The sites of enzymatic activity within the placental disc are illustrated in figs. 33, 34, 36. Three things should be noted: the reaction is restricted to the wall complex of the maternal blood passages; the syncytial trophoblast as a whole is characterized by a generalized diffuse reaction; and a thin inner segment of the wall displays an exceedingly intense reaction. Nuclear reactions are observed only after longer periods of incubation in substrate (4 hours), and are presumably artifacts (*Gomori* [1951]). It is also possible that the diffuse reaction of the syncytium is an artifact resulting from a diffusion of reaction products away from the inner zone of more intense activity during incubation. This is considered unlikely, however, for the reaction in the syncytium is apparent and uniform after only one hour of incubation, which is too short a time for appreciable diffusion to occur.

Of major interest is the exact localization of the intense enzymatic activity of the inner zone. It seems quite apparent that it does not involve the fetal syncytium, rather it appears to be concentrated in the PAS-positive membrane lying between the syncytium and the maternal endothelium. While the phosphatase preparations are not suitable for cytological discrimination of a high order, a few endothelial nuclei were found and were seen to be resting *upon* the intensely stained lamina (fig. 36) rather than within it as would be expected if the maternal endothelium were involved. It is also of interest that an intense enzymatic activity of the inner zone very generally appears to be a characteristic of the smaller placental tubules, for the intensity of the reaction diminishes as the caliber of the tubules widen nearer their connections with the larger branches of the maternal arteries and veins within the disc. In these larger vessels the inner reactive zone has disappeared altogether (figs. 34, 36), despite the fact that the PAS-positive stratum is maximally developed within them. This

distribution of an important phosphorylating enzyme within the placental disc lends support to what the architecture of the disc and common sense would strongly suggest—that the innumerable smaller tubules of the placenta are the seat of its major functions in exchange.

In conclusion it might be mentioned that the most intense phosphatase reaction in the placenta of the cat occurs in the connective tissue lamina already referred to (p. 317) which corresponds to the reactive membrane lining the syncytial tubules of *Desmodus rotundus* (cf. *Wislocki* and *Dempsey* [1946]).

Summary.

The morphogenesis of the fetal membranes and placenta of the tropical American vampire bat *Desmodus rotundus murinus* is described, together with observations on the distribution in the placenta of ribose nucleic acid, PAS-positive substances and alkaline phosphatase. The development of the placenta and membranes in the Desmodontidae most nearly resembles that of the closely related Phyllostomatidae, but differs markedly from the development of all other bats thus far described.

Following ovulation the endometrium undergoes a marked progestational reaction, which is presumably related to the interstitial implantation of Desmodus. The oviducal journey of the fertilized ovum appears to be relatively long, for the ovum develops to a full blastocyst before entering the uterus. The preimplantation development of the blastocyst is characterized by praecocious formation of entoderm and mesoderm. Subsequent to implantation, and before the appearance of the primitive streak, extra-embryonic mesoderm continues to be praecociously formed from the endoderm of the yolk sac subtending the embryonic disc. Implantation is cytolytic and completely interstitial, and occurs at the antimesometrial side of the uterus. Orientation of the embryonic disc is antimesometrial.

The definitive amnion is formed by cavitation, not by folding as in most other bats. The allantois was not observed, but the gaps between stages are small enough as to make it certain that the allantois never attains more than a rudimentary development. The yolk sac is large and permanent, but only its embryonic segment becomes vascularized. The extensive abembryonic segment remains fused with the trophoblast from which it is separated only by *Reichert*'s membrane. There is thus an extensive, permanent bilaminar omphalopleure. The growth of the fetus eventually invaginates the yolk sac-splanchnopleure, pressing it against the inner surface of the bilaminar omphalopleure, and thereby eliminating the yolk sac cavity. This relationship characterizes the "incompletely inverted yolk sac placenta".

The chorio-allantoic placenta is discoidal and labyrinthine. The placentas of most bats, including the Phyllostomatidae, are alleged to be hemochorial, but as revealed by selective staining procedures that of *Desmodus rotundus* is endotheliochorial. The placental labyrinth is separated from the basal decidua by an irregularly thickened shell of cytotrophoblast analagous to that of the human placenta. There are no accessory allantoic placental structures. The development of the allantoic placenta, and the course of the fetal and maternal circulations within it are described.

A complete *decidua capsularis* is apparent in earlier stages, but becomes incomplete later. The *decidua basalis* is prominent, although most of it has been lost by term. The parietal endometrium shows no marked decidual reaction, but is largely destroyed during the latter half of gestation.

Cytoplasmic ribose nucleic acid is found in appreciably amounts in the syncytial trophoblast, cytotrophoblast and yolk sac entoderm, but the concentration in the syncytium is far greater than elsewhere. PAS-positive substances (glycogen and glycoproteins) are widely distributed. Glycogen is restricted to the *decidua basalis* and the mesenchymal epithelium of the yolk sac splanchnopleure. Glycoproteins are found to a variable degree in nearly all constituents of the placenta and uterus, and are associated for the most part with connective tissue fibrils and ground substance. Alkaline phosphatase was observed at only two sites, the uterine surface and glandular epithelia, and the walls of the maternal lacunae within the placental disc, i.e., within the placental barrier. Characteristics of enzyme distribution within the barrier are discussed.

Résumé.

La morphogenèse des annexes fœtales et du placenta du Vampire tropical américain *Desmodus rotundus murinus* (Desmodontidae) ressemble, en général, à celle des Chauves-Souris frugivores tropicales américaines (Phyllostomatidae); mais elle diffère, sous plusieurs rapports, de celle de toutes les autres Chauves-Souris décrites jusqu'ici.

L'ovulation est suivie d'une réaction progestative prononcée de l'endomètrium. L'œuf fécondé se développe jusqu'au stade de blastocyste complet avant de quitter la trompe utérine. L'implantation est cytolytique et complètement interstitielle; elle a lieu dans l'une des deux cornes utérines, son orientation est antimésométriale.

L'orientation du disque embryonnaire est antimésométriale. Avant l'apparition de la ligne primitive, le mésoblaste extra-embryonnaire se forme précocement au pôle embryonnaire du blastocyste, peut-être à partir du trophoblaste et du disque embryonnaire, mais certainement à partir de l'endoblaste de la vésicule ombilicale. L'amnios se forme complètement par excavation comme chez les Mégachéiroptères. L'allantoïde est tout au plus rudimentaire. La vésicule ombilicale est volumineux et permanente, mais seule sa moitié embryonnaire se recouvre de mésoblaste et est vascularisée. La moitié anti-embryonnaire se segmente durant toute la grossesse jusqu'au point où elle atteint le trophoblaste et n'est séparée de ce dernier que par une membrane bien visible, la membrane de *Reichert*. Il y a donc une omphalopleure permanente, étendue et composée de deux lames. La croissance du fœtus écrase finalement la paroi vascularisée de la vésicule ombilicale contre l'omphalopleure non vascularisée et, de cette façon, ferme complètement la vésicule ombilicale et forme un type incomplet d'omphaloplacenta inversé.

L'allanto-placenta est discoïde, labyrinthique et endothélio-chorial – cette dernière caractéristique s'opposant à celle que l'on rencontre chez la majorité des Chauves-Souris, y compris les Phyllostomidés, dont le placenta, prétend-on, est hémo-chorial. Le disque placentaire est attaché, d'une façon permanente, à la caduque basale au moyen d'une couche irrégulière de cytoblaste. Les allanto-placentas accessoires sont absents.

Au début, il se forme d'une caduque capsulaire complète, mais celle-ci devient finalement incomplète. Une véritable réaction déciduale des cellules de l'endometrium est, en grande partie, restreinte à la caduque basale, mais l'endometrium pariétal, aussi bien que la caduque basale, est considérablement détruit aux stades avancés de la gestation.

C'est dans le plasmodiblaste que la concentration d'acide ribonucléique cytoplasmique est la plus élevée, mais cette concentration n'est pas inappréciable dans le cytoblaste ni dans l'endoblaste de la vésicule ombilicale. Le glycogène atteint sa plus forte concentration dans la caduque basale, mais on en rencontre aussi dans des cellules épithéliales mésenchymateuses de la splanchnopleure de la vésicule ombilicale. Les glycoprotéines abondent partout, elles sont surtout associées aux fibrilles et à la substance fondamentale du tissu conjonctif. La phosphatase alcaline est présente en abondance dans le labyrinthe placentaire où, cependant, elle est restreinte aux parois mêmes des lacunes sanguines maternelles. L'amnios ne fut pas étudié, mais l'enzyme manque dans la vésicule ombilicale vers la fin de la gestation.

Zusammenfassung.

Die Morphogenesis der Eihäute und Plazenta der tropischen amerikanischen Vampirfledermäuse *Desmodus rotundus murinus* (Desmodontidae) gleicht im allgemeinen derjenigen der nahe verwandten tropischen amerikanischen Fruchtfledermäuse (Phyllostomatidae), unterscheidet sich aber in vielen wichtigen Punkten von der bei allen anderen bisher beschriebenen Fledermäusen.

Auf die Ovulation folgt eine deutliche progestationale Reaktion der Uterusschleimhaut. Das befruchtete Ei entwickelt sich zur vollen blastozystischen Stufe, bevor es die Eileiter verläßt. Die Einpflanzung geschieht zytolytisch und vollkommen interstitiell und findet antimesometral in einem Horn des zweihörnigen Uterus statt. Die Orientierung der Keimscheibe ist antimesometral. Schon vor dem Erscheinen des Primitivstreifens bildet sich das außerembryonale Mesoderm frühzeitig im embryonalen Pol des Blastozysts, möglicherweise vom Trophoblast und von der Keimscheibe aus, aber sicherlich vom Entoderm des Dottersackes. Das Amnion wird vollkommen durch Aushöhlung gebildet, wie bei den Megachiroptera. Die Allantois ist bestenfalls rudimentär. Der Dottersack ist groß und dauernd, aber nur die embryonale Hälfte ist mit Mesoderm überzogen und wird von Blutgefäßen versorgt. Die nicht embryonale Hälfte haftet während der ganzen Schwangerschaft am Trophoblast, von dem sie durch eine deutliche *Reichert*sche Membran getrennt ist. Wir haben daher eine ausgedehnte dauernde bilaminare Omphalopleura. Durch das Wachsen des Fetus kommt die vaskularisierte Wand des Dottersackes schließlich an die avaskularisierte Omphalopleura zu liegen, wodurch die Dottersackhöhle verschwindet; so entsteht ein vollständiger Typus einer umgekehrten Dottersackplazenta.

Die allantoische Plazenta ist diskoidal, labyrinthisch, und endotheliochorial — letzteres im Gegensatz zur Mehrzahl der Fledermäuse, einschließlich der Phyllostomatiden, bei denen die Plazenta hämochorial sein soll. Die Plazentascheibe ist durch eine unregelmäßige Schicht von Zytotrophoblast an der Decidua basalis dauernd befestigt. Zusätzliche allantoische Plazenten fehlen.

Ursprünglich bildet sich eine vollständige Decidua capsularis, welche schließlich unvollständig wird. Eine wahre dezidуale Reaktion der endometrischen Zellen

beschränkt sich hauptsächlich auf die Decidua basalis, aber das parietale Endometrium wie auch die Decidua basalis werden in der späten Gravidität zum Großteil aufgelöst.

Die zytoplasmische Ribonucleinsäure ist im Syncytiotrophoblast am stärksten konzentriert; aber sie ist auch im Zytotrophoblast und im Dottersackentoderm nicht unerheblich. Glykogen findet sich am reichlichsten in der Decidua basalis, aber Ablagerungen kommen auch in den mesenchymalischen Epithelzellen der Dottersackplanchnopleure vor. Glykoproteine sind überall reichlich vorhanden, und meistens in Verbindung mit Bindegewebsfibrillen und Grundsubstanz. Alkalische Phosphatase ist reichlich im Placentalabyrinth vorhanden, wo sie sich aber auf die wirklichen Wände der mütterlichen Bluträume beschränkt. Der Amnion wurde nicht untersucht, aber gegen Ende der Schwangerschaft weist das Dottersack kein Enzym auf.

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