STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

Part V. Placentaion in the Vespertilionid Bat—Scotophilus wroughtoni (Thomas)

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The early development and implantation in Scotophilus wroughtoni has been described in an earlier paper (1949 b). It is my purpose in this paper to describe the development of the foetal membranes and the structure of the placenta in this species.

The embryology of Microchiroptera has received very little attention from the embryologists. Out of the 16 families of microchiroptera, species belonging to only a few families have been studied and even in these cases details regarding certain aspects of placentation are lacking.

Van Beneden (1888) working on the development of Vespertilio murinus (Vespertilionidae) found that the endothelium of the maternal vessels is lost in the placenta. Thus he recognised that the placenta is of the hæmochorial type. Duval (1896) redescribed the placenta of Vespertilio murinus and restated the fact of the loss of the endothelium of the maternal capillaries resulting in a direct contact of the maternal blood and the trophoblast. While van Beneden (1888) attributed a maternal origin to the trophoblast, Duval (1896) made it clear that the trophoblast is foetal in origin. Branca (1927) described the histological structure of the placenta of Vespertilio murinus and mentioned that the maternal endothelium is not completely lost but persists as an enucleate cytoplasmic membrane, so that the final structure of the placenta is not strictly hæmochorial. Gérard (1928 a) stated that in Vesperugo noctula (Vespertilionidae) the yolk-sac attains communication with the uterine lumen on the mesometrial side by the formation of an umbilico-uterine orifice. In another paper (1928 b) he mentioned that in Vesperugo noctula also the endothelium of the maternal capillaries persists as a cytoplasmic membrane as in the case of Vespertilio murinus described by Branca (1927). Kempermann (1929) working on Miniopterus schreiberii (Vespertilionidae), found the complete loss of the maternal endothelium in the placenta and also found the trophoblast directly bathed in the maternal blood—an observation quite different from that of Branca (1927) and Gérard (1928 b). Van der Sprekel (1932) in his monographic work on the persistence of the yolk-sac in the embryos of bats, stated that the yolk-sac persists in some form or other till term and also stated that it functions as a gland of internal secretion during later stages of gestation. He found the existence of a real, functional "Omphalo-placenta" in Vesperugo noctula, Rhinolophus hipposideros (Rhinolophidae) and Hipposideros (Hipposideridae). Thus he observed regarding Hipposideros CI—'Der Döttersack hat eine sehr ausgiebige Dötterplacenta entwickelt, welche fast die ganze Inneroberflasche des Uterus einnimmt, das Omphalo-chorion trägt viele Zolten, welche stark vascularisirt sind.
and in die Krypten des endometriums eintach en' (page 214). Ramaswami (1933) described the chorioallantoic placenta of Vesperugo leisleri (Vespertilionidae) to be of a hæmochorialis type and also confirmed the persistence of the yolk-sac till term, though, he mentioned, that in certain parts the yolk-sac cavity occurs as a small space, and it is absent in certain other parts during later stages of development. In a small paper, Hamlett (1934) mentioned that in the case of Molossus crassicaudatus (Molossidæ) there is a bicornuate uterus with pregnancy only in the right horn. The placenta in this species is endotheliochorial in nature. He also mentioned that the yolk-sac cavity though large does not come into direct contact with the chorion but lies free in the extra-embryonic cælon. His observations on Molossus crassicaudatus were confined to a single specimen. In a later paper (1935), he confirmed his earlier (1934) observation on Glossophaga soricina (Phyllostomidæ) that there is a simplex uterus with fundal implantation, and that the placenta is hæmochorial. He recorded also the curious fact of the formation of a decidua capsularis in Glossophaga soricina. He mentioned the early disappearance of the yolk-stalk at as early a stage as the 2.5 mm. embryo. The bilaminar omphalopleure persists till term, and the yolk-sac cavity is reduced to a minimum. Mossman (1937) found that in Nyctinomus cynocephala (Molossidæ) there is an early disappearance of the bilaminar omphalopleure and the formation of a well developed chorio-vitelline placenta which continues to bear villi up till the limb-bud stage. He described the chorio-allantoic placenta as 'endotheliochorial or hæmochorial'. Wislocki and Fawcett (1941) described the placenta in Artibeus jamaicensis parvipes (Phyllostomidæ) and stated that as in the case of Glossophaga soricina (Hamlett 1934 & 1935), the uterus is simplex, the implantation fundal, the yolk-sac large and persistent, and the chorio-allantoic placenta hæmochorial. Wimsatt (1945) in his excellent work on the placentation on Myotis lucifugus lucifugus (Vespertilionidae) described a large yolk-sac which attains great functional importance during the early stages of development. Later the chorio-vitelline placenta is destroyed by the extension of the exocælon and the consequent invagination of the roof of the yolk-sac. Wimsatt calls this condition as an example of incomplete or partial inversion of the yolk-sac. He questions the correctness of the phrase 'inverted yolk-sac placenta', since the vascular invaginated segment of the yolk-sac never comes into direct contact with the maternal tissue because of the persistence of the bilaminar omphalopleure.

2. Material and Methods

Details of collections have been given in Parts I and II and III of these studies (Gopalakrishna, 1947, 1948 and 1949 a). Pregnant uteri were fixed
in Bouin’s fixative. In cases of advanced conceptuses the uterine wall was punctured or slightly slit open with a view to allowing the penetration of the fixative. In cases of early stages of pregnancies the gravid uteri were cut at 10 μ, but in the cases of advanced pregnancy only the placenta was cut at 10 μ. Sections were stained in Ehrlich’s hæmatoxylin and counterstained with Eosin.

3. **Observations**

A. *The Implanted Blastocyst* (Plate XIII, Fig. 1)

After implantation the blastocyst can be distinguished into an embryonic and an extra-embryonic region. The former consists of an embryonic disc made up of three or four layers of cells underlined by the entoderm consisting of a single layer of fusiform cells. The embryonic disc is arched over by the amnion, which is in contact with the basal cytotrophoblast. The embryonic disc, therefore, forms the floor of the amniotic cavity.

The extraembryonic part of the blastocyst consists of the large yolk-sac which fills most of the uterine lumen and extends up to the mesometrial side of the uterus. A small part of the uterine lumen on the mesometrial side persists as small cavity, into which the uterine glands open by wide mouths. The yolk-sac cavity is lined internally by the vitelline entoderm.

On the antimesometrial and lateral sides the trophoblast has penetrated into the uterine endometrium engulfing the maternal capillaries which have lost their endothelium in certain parts (Plate XIII, Fig. 2). The penetrating trophoblast has become multi-layered and syncytial while the basal cytotrophoblast alone shows the cell boundaries. The distal wall of the yolk-sac, *i.e.*, the yolk-sac wall towards the mesometrial side, remains bilaminar.

The further development of the embryo consists of a series of events occurring in the following sequence—the differentiation of the mesoderm, the establishment of the vascular chorio-vitelline placenta, the later obliteration of this by the extension of the exocælom and the expansion of the amniotic cavity, the development of the allantois, and the final structural modifications of the chorio-allantoic placenta. While these events occur in the above-mentioned sequence, there may be a telescoping of events so that at any one stage more than one event may be taking place. I shall precede to describe these stages in the strictest order of the sequence of events.

B. **Stages leading to the formation of the Neural Groove**

(a) *Stage I*—(Plate XIII, Fig. 3).—The embryonic disc shows the formation of the neural groove and the mesoderm. The latter lies separated from the ectoderm by a clear space. The mesodermal plate is made up of a mass
of cells loosely arranged. The entoderm occurs as a thin layer of fusiform cells. The mesoderm extends laterally towards the trophoblast which is closely applied to the uterine endometrium, and invades it. The maternal vessels are surrounded by this invading trophoblast and in most parts the endothelium of the maternal vessels is lost (Plate XIV, Fig. 4).

The embryonic disc is arched over by the amnion which is closely applied to the cytotrophoblastic layer on the antimesometrial side. Only on the sides of the amniotic cavity has the amnion been reinforced by the mesoderm.

The yolk-sac cavity is large and occupies the whole of the uterine lumen except on the mesometrial side, where the persistent bilaminar omphalopleure is not in contact with the uterine tissue (Plate XIII, Fig. 3). The yolk-sac wall can be distinguished into three histologically distinct regions—the embryonic part (i.e., the roof of the yolk-sac), the lateral walls and the bilaminar omphalopleure. On the embryonic part the yolk-sac wall is made up of a layer of entodermal cells supported by two or three layers of mesodermal cells (Plate XIV, Fig. 4). As we pass towards the lateral sides, the yolk-sac wall becomes three layered because of the extension of the mesoderm between the entoderm and the trophoblast (Plate XIII, Fig. 3). The mesoderm extends only for a short distance. The lateral walls of the yolk-sac forms true placental relationship with the uterine endometrium because the trophoblast has invaded the endometrium and has surrounded the lacunae containing maternal blood. The yolk-sac entoderm is a thin layer of fusiform cells underlying the cytotrophoblast and forms the inner layer of the bilaminar omphalopleure (Plate XIV, Fig. 5).

The basal cytotrophoblast on the antimesometrial and lateral sides of the uterus migrates into the uterine endometrium. The cellular boundary of the penetrating trophoblast cannot be made out as it has become syncytial while cell boundaries are quite clear in the basal cytotrophoblast (Plate XIV, Fig. 4). The trophoblast shows the presence of the villi in some places, so that at different regions, the placental relationship is different, ranging from the syndesmochorial to the hämochorial complexity. The lateral walls of the uterus is also included in placental formation.

I have referred, in my earlier paper (1949 b), to the endometrial changes, in the uterus at the time of implantation. These changes are more pronounced and result in the formation of a loose periplacental zone, already indicating the region of separation during parturition.

(b) Stage II—(Plate XIV, Fig. 6).—In this stage the neural groove has deepened still further and the mesoderm, which was in the form of a sheet of cells underlying the embryonic ectoderm, shows the beginnings of the
formation of the cœlom. The mesoderm has extended laterally between the yolk-sac entoderm and the trophoblast up to about half of the lateral wall of the yolk-sac, so that in this part the omphalopleure is trilaminar. Angiogenesis has commenced and blood corpuscles are seen in the mesoderm. The upper part of the lateral wall of the yolk-sac is vascularised (Plate XV, Fig. 7). The mesoderm has also extended round the amnion between it and the trophoblast, thus forming an additional layer to the amnion, which is separated from the chorion. The amniotic cavity has increased in extent. The trophoblast has further invaded the maternal endometrium forming large lacunæ containing maternal blood.

The characteristic feature of this stage is the occurrence of the vascular chorio-vitelline placenta on the embryonic hemisphere of the yolk-sac together with the non-vascular yolk-sac placenta towards the lower half of the lateral wall of the yolk-sac.

The abembryonal part of the omphalopleure is bilaminar, and never establishes any placental relationship with the uterus till term.

(c) Stage III—(Plate XV, Fig. 8).—In this stage the mesoderm has extended further along the lateral wall of the yolk-sac and has reached the margin of the placental cup. The vitelline vessels have also extended further and have vascularised the major part of the lateral sides of the yolk-sac.

The changes observed in the trophoblastic syncytium in the previous stage are augmented. The junctional zone is clearly demarcated, because the uterine endometrium here has become very loose, and contains large number of spaces (Plate XVI, Fig. 9).

The uterine wall as a whole is thick on the anti-mesometrial side and gradually reduces in thickness as we pass towards the mesometrial side, finally becoming very thin on the mesometrial side. On the antimesometrial side some maternal vessels may be seen entering the placental zone.

C. Stage of Allantoic Diverticulum—(Plate XVI, Fig. 10)

The neural groove is closed and the amniotic cavity has become extensive. The allantoic rudiment occurs as a mass of cells arising from the hinder end of the embryo. The allantoic rudiment has not reached the base of the placenta.

At this stage the chorio-vitelline placenta is of great functional importance as evidenced from its structure. The whole of the lateral wall of the yolk-sac is richly supplied with fetal vessels. The roof of the yolk-sac is drawn away from the uterus as a result of the extension of the exocoelom between the somatic and the splanchnic layers of mesoderm. Only in this region
the yolk-sac placenta does not occur, because the vascular yolk-sac wall is not in contact with the trophoblast.

D. Chorio-allantoic Placentation Stages

(a) Stage I—(Plate XVII, Fig. 11).—The gap between the previous stage and this one is a little wide, since this stage is, in several respects, advanced over the previous stage in development because of—(i) the occurrence of a true chorio-allantoic placenta by the growth of the allantois with its blood vessels across the exocoelum towards the base of the placenta on the antimesometrial side, (ii) the presence of the yolk-sac placenta towards only the lower one-third of the lateral wall of the yolk-sac, the upper two-thirds being destroyed by the extension of the exocoelom; thus the chorio-vitelline placenta, and the chorio-allantoic placenta, are both functional, (iii) the reduction of the yolk-sac cavity.

The allantois is disposed towards the antimesometrial side and consists of a vascular stalk enlarged at the base of the placenta into a vesicle. The cavity of the vesicle is in continuity with the gut of the embryo. The lumen of the allantoic stalk is lined by columnar-cells, while the extra-fetal part of the allantoic lumen, including the cavity of the vesicle, is lined by fusiform cells of the allantoic mesenchyme. Thus the vesicle does not have an epithelial lining in the strictest sense of the term. The allantois carries the fetal vessels to the base of the placenta only on the antimesometrial side, the allantoic mesenchyme and the blood vessels having not yet extended to the lateral sides.

There are also some striking histological changes in the placental trophoblast. At several places the basal cytotrophoblast shows cords of cells penetrating into the syncytiotrophoblast (Plate XVII, Fig. 12), and hence in many places the cytotrophoblastic cords appear to surround the lacunae formed by the syncytiotrophoblast. As these cytotrophoblastic cords penetrate the syncytiotrophoblast they form large excavations in the syncytium and the allantoic mesenchyme and the fetal vessels enter into these excavations. The trophoblastic cords at this stage have extended up to about half the thickness of the placenta.

The periplacental layer is very clear so that the junctional zone between the syncytiotrophoblast and the decidua can be very sharply made out (Plate XVII, Fig. 12). The decidual cells are large and vacuolated, so that the tissue, as a whole, appears loose and spongy under the low power of the microscope.

As a result of the enlargement of the amniotic cavity, and the extension of the exocoelom, and also due to the increased functional importance
attained by the chorio-allantoic placenta, the chorio-vitelline placenta has partly lost its functional importance. At this stage, however, the chorio-vitelline placenta still occurs on the lower part of the lateral wall of the yolk-sac, and the placental surface is profusely supplied with vitelline vessels (Plate XVII, Fig. 12). There is one fundamental difference between the chorio-vitelline placenta and the chorio-allantoic placenta. While in the allantoic placenta the foetal mesenchyme and blood vessels actually enter the placental complex, the vitelline mesenchyme and vessels never enter the placenta. This may be partly due to the fact that in the region of vitelline placenta the cytotrophoblastic chords are not found to erode into the syncytiotrophoblast. Thus the chorio-vitelline placenta never attains the structural complexity attained by the chorio-allantoic placenta.

The yolk-sac maintains its size, but on the foetal side it narrows considerably so that its communication with the foetus is almost stalk-like.

Thus at this stage both chorio-allantoic placenta and chorio-vitelline placenta are functional.

The condition of the uterus with reference to the chorio-vitelline and the chorio-allantoic placenta has already been described. The uterine wall has become thin on the mesometrial side, and the uterine glands, which are very few, have very narrow lumina.

(b) Stage II.—The exocoelom extends laterally still further resulting in the further obliteration of the chorio-vitelline placenta. There is a distinct change in the shape of the yolk-sac. It has lost its circular outline, and has become very much smaller because the roof of the yolk-sac is pushed towards the mesometrial side by the enlargement of the amniotic cavity.

The allantoic mesenchyme has extended slightly towards the lateral sides, and the allantoic vesicle has also enlarged, and it extends in a fan-shaped manner below the placenta. The allantoic vesicle maintains its connection with the foetal gut as evidenced by an examination of the serial sections.

There are, however, marked changes brought about in the histology of the placenta. The cytotrophoblastic cords have penetrated still further into the syncytiotrophoblast, forming large excavations at the basal region of the placenta. The cavities thus formed contain the allantoic mesenchyme and foetal vessels (Plate XVII, Fig. 13). The placenta appears to be made up of columns of cytotrophoblastic cellsencircling lacunae containing maternal blood. In some parts the original syncytium is reduced to a thin membrane intervening between the maternal blood and the cytotrophoblast.

(c) Stage III—(Plate XVII, Fig. 14).—In this stage the amniotic cavity has enlarged in extent and also the exocoelom. The yolk-sac splanchnopleure
is completely separated from the placenta so that the chorio-vitelline placenta ceases to exist anywhere. The cavity of the yolk-sac has been reduced and occurs as a slit due to the invagination of the vascular roof of the yolk-sac over the persistent bilaminar omphalopleure.

The whole of the placenta is vascularised by the allantoic vessels alone, the allantoic mesenchyme having extended up to the lip of the placental cup carrying with it the foetal vessels. The cavity of the allantoic vesicle is reduced and the allantoic stalk has become solid and carries foetal blood vessels. The allantoic vesicle is therefore no more in continuity with the foetal gut.

The histological changes in the placenta consist in the further augmentation of the changes seen in the previous stage. The cytotrophoblastic villi have eroded further into the syncytiotrophoblast, creating, as they penetrate, large excavations into which the allantoic mesenchyme and foetal blood vessels enter (Plate XVIII, Fig. 15). The shape of the placenta is distinctly cup-shaped, only the mesometrial part being free from placental formation.

The yolk-sac has lost all its placental significance and has become completely replaced by the allantoic placenta.

(d) Limb-bud Stage.—This stage is characterised by the appearance of limb-buds on the foetus. I have in my collection two stages of limb-bud stage; in one there is the formation of only the fore limbs, and in the other both the limbs are formed. The histological details of the placenta in the two stages vary markedly—the latter showing more complex structure than the former. For the sake of convenience I shall describe these two stages together.

There is a progressive reduction in the yolk-sac cavity, so that in the later limb-bud stage it is reduced to a mere slit. But the distinction of the vascular splanchnopleure and the bilaminar omphalopleure is quite clear (Plate XVIII, Fig. 16). The yolk-stalk becomes a solid, vascular stalk due to the obliteration of the lumen, which connected the foetal gut with the vitelline cavity, in the early stages of development.

There is also a reduction in the extent of the cavity of the allantoic vesicle though the allantoic mesenchyme and the foetal mesenchyme and vessels have spread over the whole of the inner surface of the placental cup.

The cytotrophoblastic villi have extended almost to the margin of the placenta, where there occurs a thin layer of syncytiotrophoblast separating the cytotrophoblast from the decidua. The placenta, therefore, appears to be made up of cytotrophoblastic cords which surround lacunæ containing maternal blood, and these cords themselves are surrounded by the allantoic
mesenchyme (Plate XVIII, Fig. 17). In the stage representing the later limb-bud stage the cytotrophoblastic cords have formed a network due to the ramifications of the cords seen in the previous stage, and the meshes of the network are filled by the allantoic mesenchyme and foetal vessels (Plate XVIII, Fig. 18). The threads of the net are formed by the branching, interlacing and interconnected cords of the cytotrophoblast surrounding a thin cytoplasmic membrane (the remnant of the syncytiotrophoblast) and maternal blood.

(e) Stage of Definitive Placenta.—Parturition takes place about the last week of June or first week of July (Gopalakrishna, 1947). The stage I am describing at present belongs to a specimen collected on the 7th June. The embryo is nearly completely developed. This is further elucidated by the fact that the foetal erythrocytes have become enucleate.

The yolk-sac at this stage is reduced very much and the cavity of the yolk-sac is almost obliterated—only a little streak-like slit occurring between the bilaminar omphalopleure and the splanchnopleure. Mossman’s (1937) figure ‘C’ on Plate 9 indicates that in Vespertilionid bats the bilaminar omphalopleure shows the presence of an opening communicating the cavity of the yolk-sac with the uterine lumen on the mesometrial side. Such a communication does not exist in Scotophilus wroughtoni. I cannot also confirm the observation of van der Sprenkel (1932) of the transformation of the yolk-sac into a gland of internal secretion. I cannot say if this change comes about towards parturition.

The histological structure of the placenta has undergone marked changes. The cytotrophoblastic cords now appear to be made up of rows of flattened nuclei placed in a sheet of cytoplasm because the cell outlines have disappeared (Plate XIX, Fig. 19). The trophoblast thus appears to be made up of a complicated network of labyrinthine columns of trophoblast enclosing maternal blood, and the spaces being pervaded by allantoic mesenchyme and foetal blood vessels. The foetal erythrocytes are enucleate and they can be recognised only by their situation. The foetal and the maternal bloods are separated from each other by (i) the endothelium of the foetal vessels, (ii) the allantoic mesenchyme, and (iii) a thin layer of trophoblastic syncytium containing flattened nuclei. All these layers are foetal in origin, there being no remnant of any maternal tissue between the foetal tissue and the maternal blood. Thus at term the placenta is of the labyrinthine haemochorialis type as in the case of most of the insectivorous bats so far described.

4. GENERAL CONCLUSIONS

I have in my collection the developmental stages of two other species of insectivorous bats belonging to two different families—*Taphozoa sp.*
(Emballonuridæ) and Lyroderma lyra lyra (Megadermatidæ). I intend to
describe in a later paper, an account of the placentation of these, and to give
a comparative account of the placentation in these three families of Michro-
chiroptera-Vespertilionidæ, Emballonuridæ and Megadermatidæ. I shall,
therefore, offer here a few remarks to explain the salient features of the
placentation in Scotophilus wroughtoni.

Towards the end of implantation the trophoblast of the blastocyst has
eroded into the uterine tissue on the antimesometrial and the lateral sides.
The trophoblast can be recognised into the syncytiotrophoblast and the
cytotrophoblast. The syncytiotrophoblast arises by a proliferation of the
original primitive trophoblast (of the blastocyst). The cytotrophoblast is
the basal trophoblast underlying the syncytiotrophoblast and having distinct
cell boundaries. A decidua capsularis, as has been described in some of the
insectivorous bats belonging to the family Phyllostomidæ, such as Glossophaga soricina (Hamlett, 1934 & 1935) and Artibeus jamaicensis parvipes
(Wislocki and Fawcett, 1941), does not occur in Scotophilus wroughtoni.

As in the case of all the species of insectivorous bats so far described,
two types of placenta can be observed in Scotophilus wroughtoni also. These
are functional at two different periods of gestation—a yolk-sac placenta
which attains great functional importance during the early stages of fetal
life, and a chorio-allantoic placenta which develops later and continues to
be functional till term. Developmentally these two types of placenta may
overlap, because at certain stages the chorio-vitelline placenta and the
chorio-allantoic placenta are both present. But as the chorio-allantoic
placenta becomes more and more elaborate the chorio-vitelline placenta
diminishes in functional importance. Actually there is a progressive replace-
ment of the chorio-vitelline placenta by the chorio-allantoic placenta.

A. Yolk-sac Placenta

(a) Morphogenesis of the Yolk-sac.—During the course of the develop-
ment the yolk-sac undergoes a number of important morphological changes,
and each phase is characterised by a number of histological changes. All
these changes, which occur during successive periods of fetal life, start from
the embryonic segment of the yolk-sac and progressively pass on to the
abembryonic pole. Thus at any particular stage in development there may
be differences in the histological details in the different regions of the yolk-sac.
The only region of the yolk-sac, which does not undergo much change, is the
bilaminar omphalopleure at the abembryonic region, which remains more or
less unaltered till term.

The yolk-sac which, at the end of implantation, was lined by the tropho-
blast and the underlying entoderm (Fig. 1) becomes trilaminar due to
the extension of the mesoderm between these two layers, beginning from the embryonic segment (Figs. 3 & 10). The mesoblast extends up to the margin of the placental cup. At first there is only the mesoderm occurring between the trophoblast and the entoderm, but soon angioblasts make their appearance and the non-vascular trilaminar omphalopleure becomes vascular, starting from the embryonic segment and progressively extending to the placental lip (Figs. 3, 7, 9 & 12). Thus the non-vascular yolk-sac placenta becomes the vascular yolk-sac placenta. Finally, due to the extension of the exocælom, the vascular trilaminar omphalopleure splits up into an outer somatopleure and an inner splanchnopleure (Figs. 3, 10 & 12). The somatopleure underlies the placental trophoblast while the splanchnopleure carrying the vitelline vessels covers the vitelline entoderm. The exocælom, therefore, as it extends from the embryonic segment, destroys the vascular yolk-sac placenta from that region. Further change in the gross morphology of the yolk-sac is the result of the invagination of the vascular roof of the yolk-sac over the bilaminar omphalopleure due to the expansion of the amniotic cavity and the exocælom. This results in the reduction of the extent of the yolk-sac cavity (Figs. 12 & 14). In advanced conceptuses the vitelline cavity occurs as a small space bounded by the bilaminar omphalopleure on the mesometrial side and the vascular splanchnopleure on the antimesometrial side.

(b) The Non-vascular Yolk-sac Placenta.—The yolk-sac wall attains placental relationship with the uterus at the end of implantation. At first the omphalopleure is throughout bilaminar being formed by the trophoblast and the entoderm. Only on the mesometrial side the yolk-sac wall does not contact the uterine tissue and remains as such till term. The placental trophoblast can be recognised into two regions—the superficial cytotrophoblast which is distinctly cellular, and made up of columnar cells, and the penetrating syncytiotrophoblast which does not show any cell boundaries and progressively penetrates deeper into the uterine endometrium (Figs. 5 & 9). The syncytiotrophoblast is derived by the rapid proliferation of the cytotrophoblast, so that a clear boundary between the two cannot be made out. As the syncytiotrophoblast penetrates more and more into the endometrium, the endothelium of the maternal capillaries is finally lost. Large trophoblastic lacunæ containing maternal blood appear.

The yolk-sac entoderm is made up of a single layer of fusiform cells (Figs. 5 & 16).

(c) The Vascular Yolk-sac Placenta.—As the mesoblast extends between the trophoblast and the entoderm it converts the bilaminar omphalopleure into a trilaminar omphalopleure. The mesoblast extends from the embryo
up to the lip of the placental cup. Angioblastic tissue soon appears in the mesoblast, and the area vasculosa extends right up to the lip of the placental cup. Thus the vascular chorio-vitelline placenta is produced, which is of some physiological importance till the chorio-allantoic placenta is formed.

Histologically the chorio-vitelline placenta shows the separation of the foetal and maternal bloods by the foetal endothelium, the yolk-sac mesenchyme, the cytotrophoblast, and the syncytiotrophoblast. The maternal endothelium will have been lost by the time the yolk-sac wall becomes vascular.

As a result of the extension of the exocoelom and the amniotic cavity the vascular splanchnopleure of the yolk-sac is separated from the somatopleure and the chorio-vitelline placenta becomes obliterated. Further the increased functional importance of the chorio-allantoic placenta reduces the significance of the yolk-sac as a placental structure. Thus the yolk-sac placenta is functional for a short period in embryonic life.

The yolk-sac placenta of Scotophilus wroughtoni therefore resembles the yolk-sac placenta of Vesperugo leisleri (Ramaswamy, 1933), and Myotis lucifugus lucifugus (Wimsatt, 1945). As in these cases the distal wall of the yolk-sac remains distinctly bilaminar. There is nothing comparable to the 'Inverted yolk-sac placenta'. The distal wall of the yolk-sac does not get perforated, as is apparent from Mossman's (1937) figure of the yolk-sac of the Vespertilionid bat (Plate 9, Fig. C. Mossman, 1937). So the yolk-sac cavity does not become continuous with the uterine lumen.

B. Chorio-allantoic Placentation

I have given a fairly detailed account of the penetration of the endometrium by the trophoblast and the formation of the trophoblastic lacunae in my earlier paper (1949b). Thus, at the end of implantation, the chorionic placenta will have been established and this is already of a haemochorial complexity in most places. The further structural changes in the placenta consist in the vascularisation of this chorionic complex by the foetal vessels through the allantois resulting in the formation of a labyrinthine haemochorial placenta.

As development proceeds the thickness of the placenta increases due to the further invasion of the uterine endometrium by the syncytiotrophoblast. Secondly, the shape of the placenta becomes changed from the original cup-shaped condition to a hollow saucer-shaped condition. More than the gross morphology, the histological structure of the placenta undergoes marked changes. As the allantois grows across the exocoelom towards the placenta, it carries with it foetal vessels and the mesoderm. The latter gradually spreads
out towards the margin of the placental cup carrying along with it the allantoic vessels. Simultaneously with the spreading of the mesoderm on the base of the placenta, the cytotrophoblastic layer becomes active and begins to send in, by proliferation, cords of cells into the syncytiotrophoblast (Figs. 4, 7, 12 & 14). These cords as they penetrate the syncytiotrophoblast towards the decidual region, produce large excavations in the syncytiotrophoblast, into which the allantoic mesenchyme and the fœtal blood vessels enter. The cytotrophoblastic cords at first occur as columns of cytotrophoblastic cells surrounding the syncytiotrophoblast and the lacunæ (Fig. 17), but later by the formation of lateral branches, the whole placenta appears like a network of tubes (Fig. 18), and the meshes are occupied by the allantoic mesenchyme and the fœtal vessels. The threads of the net are tubular and formed by a thin layer of syncytiotrophoblast supported by a layer of cytotrophoblast. The lumina of the tubes contain maternal blood. The cytotrophoblast does not come into direct contact with the maternal blood.

The further reorganisation of the placenta consists in the elimination of the cytotrophoblastic cords which occurs as a layer between the allantoic mesenchyme and the syncytiotrophoblast. The cells of the cytotrophoblast gradually lose their cellular boundaries, and in later stages only flattened darkly staining nuclei occur in rows forming a wall to the fœtal mesenchymal tissue. Thus at final stages the placenta is made up of ramifying tubules of trophoblast occurring in the allantoic mesenchyme and fœtal blood vessels. The maternal blood occurs inside the trophoblastic tubules.

Thus when fully formed, the placenta is labyrinthine and hæmochorial and the tissues that separate fœtal and maternal bloods are all fœtal in origin—the maternal endothelium and the endometrial tissue being destroyed at a very early stage of development.

5. Summary

1. The yolk-sac which is disposed towards the mesometrial side establishes placental relationship with the uterus. The yolk-sac placenta is at first non-vascular and later becomes vascular.

2. As development proceeds the extension of the exocœlom and the expansion of the amniotic cavity results not only in the obliteration of the yolk-sac placenta, but, in the invagination of the yolk-sac roof over the distal bilaminar omphalopleure.

3. The allantois is disposed towards the antimesometrial side. The allantois gradually spreads towards the lateral sides below the placenta and progressively replaces the yolk-sac placenta.
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4. The changes involved in the formation of the definitive labyrinthine placenta are: (i) The penetration of the syncytiotrophoblast by the trophoblastic chords, (ii) the invasion of the cavities thus formed by the allantoic mesenchyme and foetal vessels, (iii) the loss of the cell boundaries in the cytotrophoblastic cords, which therefore occurs as a network of cytoplasm with flattened nuclei embedded in rows.

5. The final placenta is therefore of the labyrinthine haemochorial type.

6. Acknowledgement

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7. References

* References marked in asterisk were not available in original.

7. ———— “Studies on the embryology of Microchiroptera. Part III.—The histological changes in the genital organs and accessory reproductive structures during the sex-cycle of the Vespertilionid bat—Scotophilus wroughtoni (Thomas),” Ibid., 1949 a, 30 B.
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(ABBREVIATIONS used.—ALL. d., Allantoic diverticulum; ALL. ve., Allantoic vesicle; AM., Amnion; AM. c., Amniotic cavity; BI. om., Bilaminar omphalopleure; EXO., Exocelom; F. V., Fœtal blood vessel; M. Bl., Maternal blood vessel; PI., Placenta; SPL., Yolk-sac splanchnopleure; Y. S., Yolk-sac cavity.)

N.B.—The arrow points towards the mesometrial side wherever it is drawn. All figures are photomicrographs, and magnifications are approximate.

EXPLANATION OF PLATES

PLATE XIII

FIG. 1. The Implanted blastocyst. The amniotic cavity is formed and the trophoblast has penetrated the uterine endometrium on the antimesometrial side. On the mesometrial side it is free so that a part of the uterine lumen persists, and large mouthed uterine glands are seen to open into this part of the uterine lumen. × Circa 25.

FIG. 2. A part of the antimesometrical wall of the uterus of the same specimen as seen in the previous figure enlarged (a part of the rectangle in Fig. 1). Note the trophoblast penetrating into the endometrium. The large lacunae are filled with maternal blood. Note the villi-like cords of the cytotrophoblast entering the endometrium, and in deeper layers it becomes syncytial. × Circa 135.

FIG. 3. Stage of early neural groove formation. The mesoderm has differentiated and has extended up to about the middle of the lateral wall of the yolk-sac. × Circa 35.

PLATE XIV

FIG. 4. A part of the blastocyst in Fig. 3 (part of the rectangle near the embryonic disc), magnified. The endoderm, made up of a single layer of flattened cells, can be clearly seen underlying the mesoderm. Note the penetration of the trophoblast into the endodermium and the formation of large lacunae containing maternal blood and surrounded by the trophoblast. Deeper to the layer of the syncytiotrophoblast, the uterine endometrium shows a layer of loose tissue full of spaces. × Circa 290.

FIG. 5. Lower part of the lateral wall of the yolk-sac in Fig. 3 (part of the lower rectangle enlarged). The mesoderm does not occur in this region. A part of the free bilaminar omphalopleure is also included in the figure. × Circa 170.

FIG. 6. Later neural groove stage. Only the embryonic disc is shown to indicate the formation of the celom in the mesoderm which occurs on the two sides of the middle line. × Circa 150.

PLATE XV

FIG. 7. A part of the lateral wall of the yolk-sac of the specimen shown in Fig. 6. Note the extensive penetration of the trophoblast into the uterine endometrium, and the complete loss of the endothelium of the maternal vessels. The trophoblast is syncytial all round, except the basal cytotrophoblast where cell boundaries are distinct. Angioblasts can be seen below the layer of the cytotrophoblast. A large lacuna containing maternal blood is included in the figure. × Circa 275.
Fig. 8. Late stage in the formation of the neural groove. The mesoderm shows the formation of the myotomes, and the fetal vessels are beginning to be formed. × Circa 125.

Plate XVI

Fig. 9. A part of the lateral wall of the uterus of the specimen shown in Fig. 8. The yolk-sac wall shows the presence of fetal blood vessels. The syncytiotrophoblast has penetrated still further. Cell boundaries are very clear in the basal cytotrophoblast. Below the placental layer the uterine endometrium shows a layer full of intercellular spaces. × Circa 185.

Fig. 10. Longitudinal vertical section of an embryo with closed neural groove. Note the vascularisation of the yolk-sac up to the placental lip. The allantoic rudiment occurs as a slight diverticulum on the posterior part of the embryo (towards the right hand side of the figure). × Circa 35.

Plate XVII

Fig. 11. Embryo showing the allantois carrying fetal vessels to the placenta on the antimesometrial side. The exocelom has destroyed the yolk-sac placenta for some distance in the embryonic segment. So the yolk-sac placenta occurs only on the lateral sides. × Circa 35.

Fig. 12. Part of the gestation sac of the specimen shown in Fig. 11, showing the yolk-sac placenta and a part of the allantoic placenta. Note the presence of the cytotrophoblastic chords penetrating the syncytiotrophoblast, and the penetration of the allantoic mesoderm and fetal vessels into the excavations so formed by the cytotrophoblast. The decidua-syncytial junction is quite clear. The zone of separation of the parturient is already foreseen in this stage by the formation of a cleft-like space which occurs all round the placental region. × Circa 140.

Fig. 13. Part of the placenta of a specimen described in Stage II of the text. The syncytiotrophoblast is invaded by the cytotrophoblastic cords, the allantoic mesenchyme and the allantoic vessels. × Circa 160.

Fig. 14. Part of the uterus containing an embryo wherein the yolk-sac placenta is completely destroyed as a result of the extension of the exocelom. The cavity of the yolk-sac is reduced to a slit-like space because of the expansion of the amniotic cavity. The bilaminar omphalopleure is persistent. × Circa 35.

Plate XVIII

Fig. 15. Part of the placenta of the specimen shown in Fig. 14. Note the extensive penetration of the allantoic mesenchyme and the fetal vessels into the placenta. × Circa 130.

Fig. 16. A part of the amnion and a part of the yolk-sac are shown in the figure. The roof of the now reduced yolk-sac carries the vitelline vessels. × Circa 240.

Fig. 17. A part of the placenta in the early limb-bud stage. The placenta is made up of tubules of cytotrophoblast surrounding a thin layer of syncytiotrophoblast; the tubules contain maternal blood corpuscles. The tubules are separated by allantoic mesenchyme containing fetal vessels. × Circa 150.

Fig. 18. Placenta in late limb-bud stage. The placenta has become typically labyrinthine and haemochorial. The spaces of the labyrinth are filled with allantoic mesenchyme and fetal vessels. The fetal erythrocytes still have nuclei. × Circa 105.

Plate XIX

Fig. 19. Placenta in late stage from a specimen collected on 7th June. The placenta is made up of a complicated trophoblastic network. The trophoblast has become syncytial. The fetal vessels are found between the trophoblastic cords of the net. The fetal erythrocytes have lost their nuclei. × Circa 130.