OBSERVATIONS ON THE DELIVERED PLACENTA AND FETAL MEMBRANES OF THE AARDVARK, *ORYCTEROPUS AFÉR* (PALLAS, 1766)

by

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SUMMARY

A macroscopical description is given of the delivered placenta and foetal membranes of an Aardvark. Special attention is paid to the umbilical cord and the relative position of the foetal membranes at the places where these attach to the placenta. Amniotic pustules were found on the umbilical cord and on the antimesometrial side of the amnion. Many dark-coloured spots were found on the villous part of the placenta.

A histological investigation was made of the umbilical cord and the amniotic pustules. Groups of longitudinally arranged smooth muscle cells in that part of the umbilical cord that had been closest to the foetus, suggested the presence of a musculus sphincter umbilicalis. The amniotic pustule on the antimesometrial side of the amnion contained many hair follicles. Hence a short commentary is given on the differentiation of the amniotic ectoderm. The dark spots on the placenta histologically appeared to be the remnants of the chorionic vesicles.

INTRODUCTION

Only a few studies have been made on the placenta and fetal membranes of the Aardvark. Horst (1949) described a very early stage of placentation, while Turner (1876) and Mossman (1957) studied pregnant uteri near term. Data concerning the delivered placenta are not available in the literature. On the 14th of August, 1969, an Aardvark was born in Artis, the zoological gardens of Amsterdam, and we were very fortunate to receive the afterbirth.

METHOD

At birth the placenta was totally inverted, so that we received it with the foetal side turned outwards. After we had brought it into its original, intra-uterine position, we found that only one side of the chorionic sac had been torn during birth.

After sectioning the chorionic sac along its long axis (fig. 2), we measured the length of the umbilical cord and then placed the placenta with the membranes in fixative (5⁰/₀ formol). After fixation, the following parts were embedded in paraffin for histological investigation:

(a) small segments of the 67 cm long umbilical cord, taken at distances of 35.5 cm, 39.0 cm and 47.0 cm from the inner surface of the placenta; (b) the free end of the umbilical cord; (c) a villous part of the placenta (fig. 2); (d) a villous part of the placenta with a brown-coloured spot (fig. 2); (e) a piece of the chorionic membrane, where the placenta was interrupted and where a brownish pustule was seen at the foetal side (fig. 3 arrow); (f) some pieces of the amniotic covering of the umbilical cord at the point where the umbilical blood vessels diverged.

Paraffin sections (5—10 μ) of the umbilical cord were stained by the method of Goldner, combined with resorcin fuchsin, and also in orceïn to show the elastic tissue. Sections of the villous parts of the placenta were stained by the method of Goldner, P.A.S., H.E., and iron-haematoxylin.

MACROSCOPICAL DESCRIPTION

1. The umbilical cord

Since the birth of the Aardvark was not observed, we do not know whether the umbilical cord broke spontaneously during birth or whether it was bitten by the mother. The latter seems very doubtful in this species in view of the development of the teeth, although the tearing of the umbilical cord by the mother of a Two-toed Sloth, *Choloepus didactylus* (cf. Veselovsky, 1966) and eating of

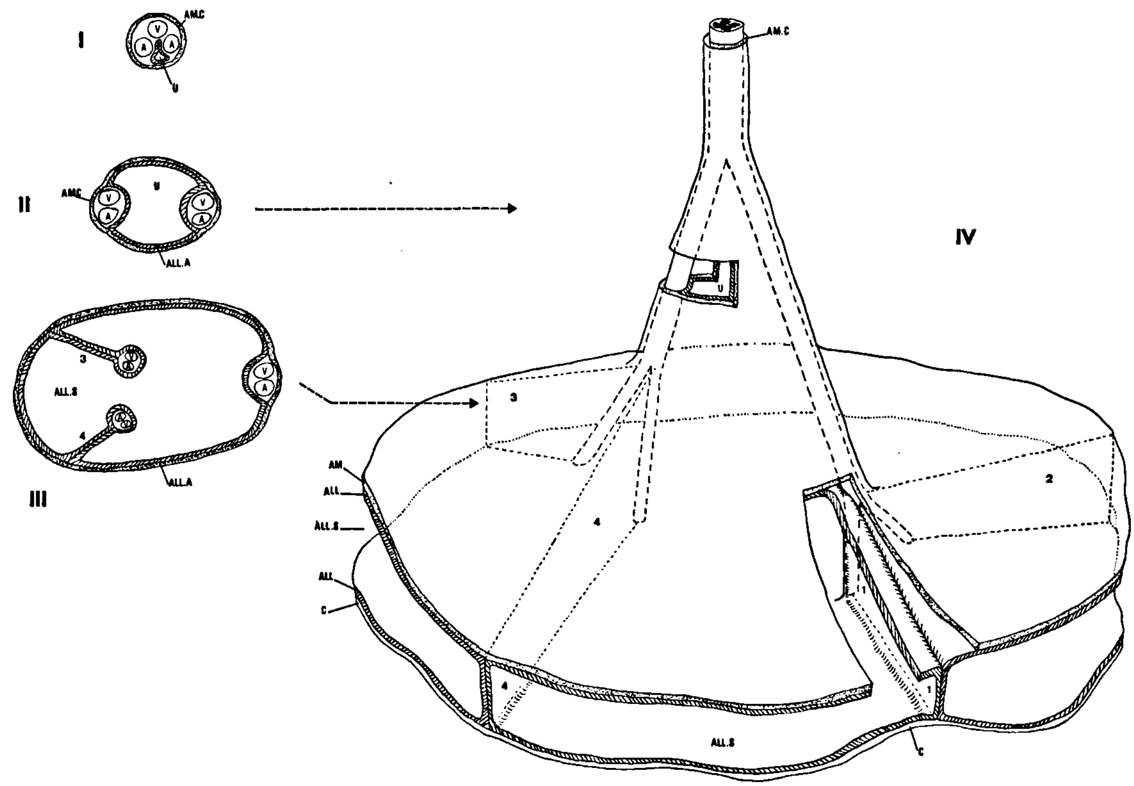


Fig. 1. Schematic drawing of the foetal membranes and the umbilical blood vessels at the place where the latter attach to the placenta (IV). At some places, parts of the foetal membranes have been removed to show their relative positions.

I: cross-section of the umbilical cord at a distance of about 47 cm from the inner surface of the placenta.

II: cross-section of the umbilical cord at the level indicated (arrow).

III: cross-section of the umbilical cord at the place where the urachus extends into the allantoic space (level of section indicated by arrow).

A.: arteria umbilicalis; ALL.: allantois; ALL.A.: allantoamnion; ALL.S.: allantoic space; AM.: amnion; AM.C.: amniotic covering of the umbilical cord; C.: chorion; U.: urachus; V.: vena umbilicalis. 1, 2, 3, and 4: allantoic septa (these numbers correspond to those of Mossman, 1957).

the placenta by the mother of an Ant-eater, *Myrmecophaga tridactyla* (cf. Honigmann, 1935) are also surprising phenomena.

However, we did not find any damage of the umbilical cord tissue at its free end, as would be expected if the cord had been chewed. The umbilical cord measured 67 cm from its free end to its attachment on the placenta. Mossman (1957) measured an umbilical cord of 27 cm (total length of the fetus: 43 cm, C.R. length: 24 cm) and Turner (1876) measured one of 57 cm (total length of the fetus: 51 cm). The umbilical cord was flattened at its free end and only three blood vessels could be seen within the cord, one vein and two arteries. These three vessels lay beside each other like they do in the umbilical cord of the horse at a distance of about 8 cm from the umbilical skin (Hauptmann, 1911).

More proximal to the placenta, the three blood vessels were arranged triangularly with the urachus in the centre (fig. 1 I). At a distance of about 40 cm from the placental inner surface, the single umbilical vein divided into two branches. Closer to the placenta, the umbilical blood vessels diverged into two pairs, each pair consisting of one artery and one venous branch. Between these pairs of blood vessels the urachus formed a large funnel (fig. 1 II). About 12 cm from the placenta, this funnel suddenly expanded enormously to a cone-shaped structure. At this point the amniotic covering of the umbilical cord fused with the wall of the allantoic duct, forming the allantoamnion. At two opposite places, one artery and one venous branch descended together to the placenta, one set of vessels dividing again about 6 cm from the inner surface of the placenta (fig. 1 III), the other about

2 cm from the placenta's inner surface. The umbilical cord was thus attached to the placenta by four sets of blood vessels (fig. 1 IV). The space between these four sets of blood vessels enabled the allantoic sac to expand between the amnion and the entire chorion (placenta and chorion laeve). As can be seen in fig. 3, the amniotic covering of the umbilical cord was dark in colour, which was due to cornification of the amniotic epithelium, as we shall describe in detail in the next section.

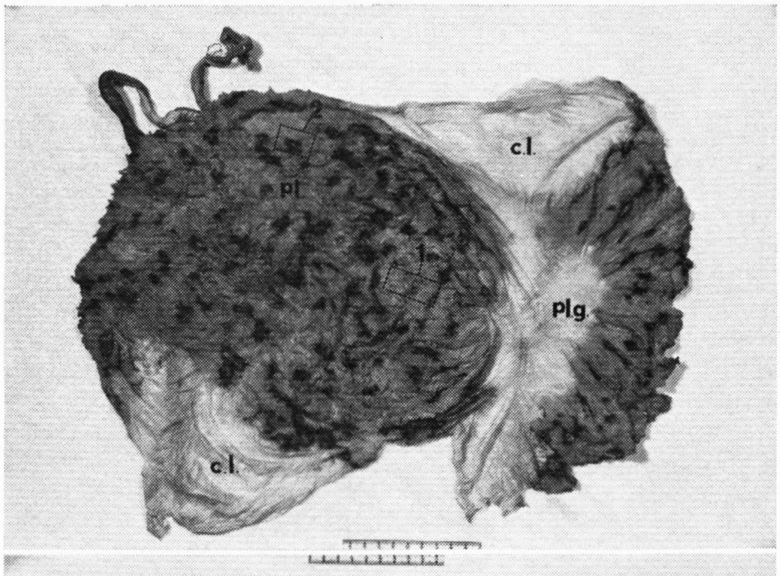
2. The placenta and fetal membranes

The Aardvark is an uniparous mammal with an uterus duplex. The entire conceptus develops in one uterus, while the other one remains nonpregnant. In the literature the Aardvark placenta is described as a placenta zonaria incompleta, but in our view one might better call it an incomplete placenta diffusa (fig. 2), for its bears a morphological resemblance to the placenta of the Equidae and the Rhinoceros, rather than to any type of zonary placenta of the Carnivora. The length of the placental girdle was 36 cm, the gap in the girdle was 4.5 to 7 cm, and the girdle was 22 to 28 cm in width. There was also a distinct chorion laeve on each side of the placental girdle, so that the complete chorionic sac was oval in shape. The distance between the two outer poles of the chorion laeve measured about 35 cm. According to Turner (1876) and Mossman (1957), who sectioned pregnant uteri, the gap in the placental girdle has an antimesometrial position in the uterus. In the gap, as well as in the chorion laeve, vascularization could distinctly be seen. The maternal side of the placental girdle exhibited many dark brown spots, which were almost equally distributed over its surface (fig. 2). Such large spots are not described in the literature, either from the Aardvark placenta or from any other type of placenta. We shall return to this problem in the next section.

The chorionic villi were orientated in ridges which ran over the length of the placental girdle. Each villus formed many secondary villi which had a flattened appearance. Between the ridges many small villi with an irregular orientation were seen on the chorionic membrane. In the central part of the dark spots, no large villi could be observed macroscopically, but in the border zone large branched villi occurred which were partly dark in colour and partly of the same hue as normal villi. Along the two sides of the placental girdle only small villi could be seen.

On the fetal side of the placenta, the allantois had completely fused with the chorionic membrane, forming the allantochorion (fig. 3). We did not find a deeply grooved fetal surface of the placenta, as described by Mossman (1957). Only some small lobes could be seen. The dark spots of the villous outer surface could be seen through the allantochorion. As described above, the umbilical cord was attached to the inner surface of the placenta at four places. Between these points of attachment the allantoic sac expanded in four lobes between the chorion and the amnion. Where two of these allantoic lobes touched each other, the allantoic membranes were fused to form transparent allantoic septa (figs. 1 and 4). In the middle of the gap in the placental girdle, all fetal membranes were fused together, the allantoic cavity and allantoic septa being totally obliterated. The course of the allantoic septa along the placental inner surface up to this antimesometrial point was exactly as described and depicted by Mossman (1957). We therefore decided to use the same numbers for the four allantoic septa (figs. 1 and 4) as Mossman used in his schematic drawings. We did not find a distinct vascularization of the allantoamnion and allantoic septa. There was a white strand of tissue visible in the allantoamnion running from the umbilical cord up to the point where all fetal membranes were fused together (fig. 3). This was probably the vitelline duct. Its course in the allantoamnion between allantoic septa nr. 3 and nr. 4 was in full accordance with the findings of Mossman (1957) (see fig. 1).

We found some amniotic pustules (pearls) on the epithelial covering of the umbilical cord, at the place where the umbilical blood vessels diverged. These pearls were oval in shape and had a long or, less frequently, a very short stem (fig. 5). They were all situated in the neighbourhood of the vitelline duct, which was immediately beneath the amniotic covering. There was also a brown coloured protrusion of the amniotic surface at exactly the point where all fetal membranes were fused together and the vitelline duct ended. (arrow in fig. 3). It was very interesting to find these pearls on the amnion of the Aardvark, for they are known from only a few species. Hill & Burne (1922) noticed amniotic pustules on the umbilical cord of the lemurid, *Chiromys madagascariensis* (syn. *Daubentonia*) also at the place where amnion and allantois fuse together.



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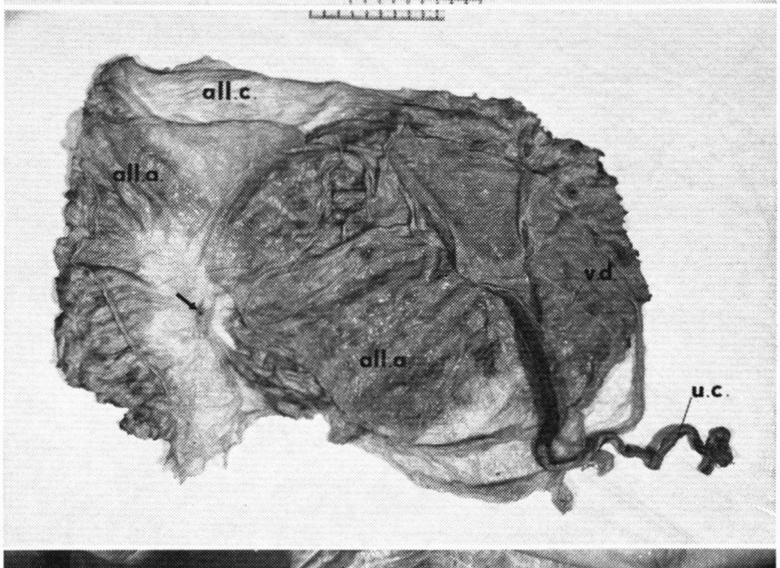

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Fig. 2. Maternal surface of the placenta and chorion laeve, after cutting the chorionic sac along its long axis. Note the dark spots on the placental girdle.
c.l.: chorion laeve; pl.: villous part of the placenta; plg.: gap in the placental girdle. Squares 1 and 2 were sectioned for histological examination. Scale in cm.
Fig. 3. Foetal surface of the placenta and foetal membranes, after cutting the chorionic sac along its long axis. Note the dark covering of the umbilical cord and the small dark coloured protrusion on the allantoamnion (arrow).
all.a.: allantoamnion; all.c.: allantochorion; u.c.: umbilical cord; v.d.: vitelline duct. Scale in cm.
Fig. 4. Photograph of two allantoic septa (2 and 3) at their origin near two pairs of umbilical blood vessels (arrows), where the latter attach to the foetal surface of the placenta. The dark area in the middle of the picture represents the opening between two pairs of blood vessels through which the allantoic space narrows into the urachus.
all.a.: allantoamnion; all.c.: allantochorion.

Naaktgeboren & Zwillenberg (1961) investigated the umbilical cord and amniotic membranes of many mammals and found many different types of amniotic pustules, but only in whales and ungulates. Since the Aardvark has a well developed allantois, our finding is in full accordance with Naaktgeboren & Zwillenberg's (1961) conclusion, that amniotic pearls are present only in species with a well developed allantois.

HISTOLOGICAL FINDINGS

1. The umbilical cord and its vessels

There is much confusion in the literature about the nomenclature of the different layers in the walls of the umbilical blood vessels. Therefore we decided to use the following classification:

Tunica intima: this layer comprises the endothelial lining of the lumen and a single elastic tissue membrane just beneath the endothelium. Muscle cells do not belong to this layer.

Tunica media: this layer forms the muscular part of the wall, containing smooth muscle cells, collagenous connective tissue and elastic fibers.

Tunica adventitia: this layer consists of the connective tissue around the muscular wall and contains many very small elastic fibers.

a. The free end of the umbilical cord

In cross sections of the portion of the umbilical cord which had been closest to the fetus, the three umbilical blood vessels lay beside each other, with the very small urachus between the two arteries. The blood vessels and the urachus were embedded in a very dense connective tissue of collagen fibers, containing many small blood vessels and capillaries of unknown origin. Thus a Wharton's jelly, usually described as a gelatinous or semi-fluid tissue, is not present in the umbilical cord of the Aardvark. Two small blood vessels with obliterated lumina and degenerated walls were found beside the umbilical artery in the middle of the cord. These were probably the remnants of the vitelline blood vessels. Small groups of cross-sectioned longitudinal muscle fibers were found at many places, embedded in the connective tissue of the cord, even at a considerable distance from the tunica adventitia of the blood vessels (fig. 6). It would be interesting to investigate the umbilicus of the Aardvark, for these groups of muscle fibers could be offshoots of muscle cells in the umbilicus, forming an umbilical sphincter. According to Parry (1954) and Mathur (1955) an umbilical sphincter appears only in ungulates and some rodents.

The wall of the constricted umbilical arteries was about 0.8 mm thick. Beneath the endothelial lining of the lumen, there was a very thick inner elastic fenestrated membrane, consisting of a network of thick elastic fibers with a spirally orientation. The inner part of the tunica media contained many thin elastic membranes with the same architecture as described for the inner elastic membrane. These thin membranes also ran parallel to the star-shaped outline of the arterial lumen, which was almost completely obliterated. Between the thin elastic membranes the muscle cells were longitudinally arranged. In the outer part of the tunica media, thick elastic fibers were arranged longitudinally in almost concentric layers. The connective tissue of the tunica adventitia could clearly be distinguished from the tunica media and from the matrix of the cord by the density of its many thin elastic fibers.

The lumen of the umbilical vein was flattened, not star-shaped in appearance. The wall of the constricted umbilical vein was about 0.7 mm thick. Beneath the endothelial lining there was a network of very thin elastic fibers. This network extended into the inner part of the tunica media. No thick inner elastic membrane could be distinguished. The architecture of the tunica media and tunica adventitia was almost the same as described for the umbilical arteries. This is a well-known feature

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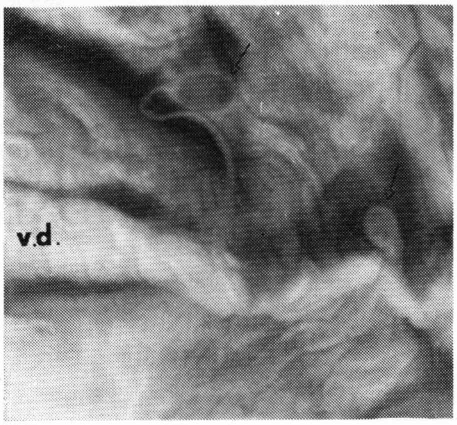

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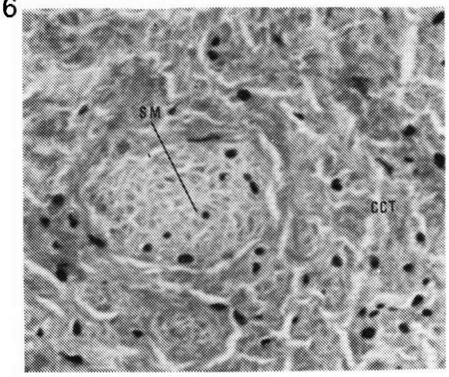

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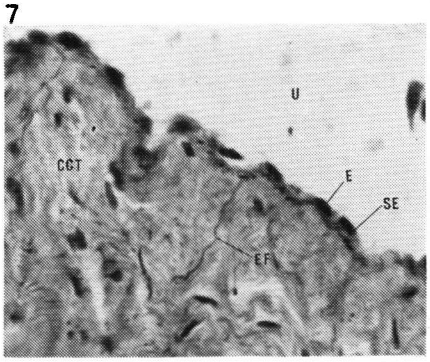

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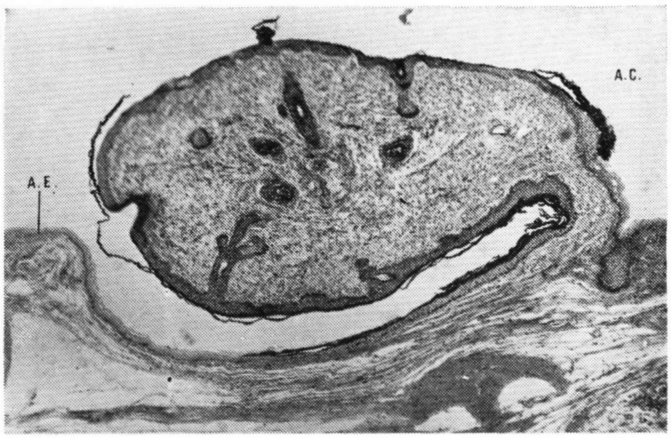

Fig. 5. Two amniotic pearls of the umbilical cord (arrows), one with a short, the other with a long stem. They are placed just beyond the vitelline duct (v.d.), which occurs beneatch the amnion (c. 12½x).

Fig. 6. Cross-section of longitudinal muscle fibers arranged in groups between the collagenous matrix at the free end of the umbilical cord (c. 285x).

S.M.: smooth muscle fibers; C.C.T.: collagenous connective tissue (stained by the method of Goldner).

Fig. 7. Detail of the wall of the urachus at a distance of about 39 cm from the placental inner surface. Description in the text (c. 420x, stained with orcein).

C.C.T.: collagenous connective tissue; E.: epithelial lining of the urachus; E.F.: thick elastic fiber; S.E.: subepithelial network of thin elastic fibers; U: lumen of the urachus.

Fig. 8. Cross-section of the amniotic protrusion at the antimesometrial side of the placenta where all fetal membranes have fused (cf. fig. 3, arrow). Note the cornuated amniotic epithelium (A.E.) and the hair follicles in various states of development. A.C.: amniotic cavity (c. 37½x, stained by the method of Goldner).

in the extra-embryonic parts of umbilical veins in mammals.

The amniotic covering of the umbilical cord consisted of a stratified epithelium which was cornuated over almost the whole surface.

b. The umbilical cord 47 cm and 39 cm from the placenta's inner surface

At 47 cm from the placenta, the umbilical blood vessels were arranged in a triangle. (fig. 1, I). Between the blood vessels, the urachus had largely expanded, with a flattened lumen about 3 mm in cross-section. This lumen was lined by a single epithelial layer or, at some places, by a transitional epithelium. The architecture of the walls of the blood vessels was the same as described above. The amniotic covering consisted of a stratified, cornuated epithelium.

At a distance of 39 cm from the placenta's inner surface, there were four blood vessels in the umbilical cord, the umbilical vein being divided into two branches. Two almost identical portions of the umbilical cord were separated from each other by the large allantoic duct (fig. 1, II), which had a single, flattened, epithelial lining. In sections stained with orcein or resorchin-fuchsin, a particular arrangement of elastic fibers was found in the connective tissue around the allantoic duct (fig. 7). Immediately beneath the epithelial lining elastic tissue fibers formed a single networked membrane. Very thick elastic fibers, which ran perpendicular to the surface of the lumen through the surrounding collagenous connective tissue, adhered to the subepithelial networked membrane and more peripherally to a broad zone of thin elastic tissue fibers.

The inner part of the tunica media of both arteries and veins contained more connective tissue and less muscular elements than in the sections described above. The muscle fibers in the inner part of the tunica media of the venous branches also exhibited a more circular arrangement. These architectural differences were probably partly due to the less constricted condition of the blood vessels at this level. The amniotic covering of the umbilical cord consisted at most places of a stratified epithelium, more or less cornuated, while at some places there was only one single epithelial layer.

2. Amniotic pearls

As mentioned above (page 156), there was a placental gap at the antimesometrial side, where all membranes were fused. In cross sections of this gap, all the membranes appeared to have contributed to the formation of a mass of connective tissue, richly supplied with blood vessels. The termination of the vitelline duct here appeared as a denser strain of connective tissue, containing large blood vessels, the walls of which were degenerate. The dark spot seen on the amnion (fig. 3, arrow) appeared merely to be a fold of the amniotic epithelium and the underlying connective tissue, for all serial sections (fig. 8) showed an ovally-shaped mass of tissue, connected with the underlying amnion by a short stem. The epithelium of this amniotic pearl was multilayered and totally cornuated, while even hair follicles in almost every state of development could be seen in the underlying connective tissue, which formed the greatest part of this pearl. This connective tissue was well vascularized. The epithelium of the amnion lying immediately beneath this pearl was also totally cornuated while the surrounding parts of the amnion contained a multilayered, incompletely cornuated epithelium. Schinckel (1962) describes hair follicles with completely differentiated hairs from the umbilical cord and amnion of the sheep. We were unable to locate any other references in the literature to the presence of hair follicles in the amnion. Schramm (1962, a, b) states that the differentiation of the ectoderm into a skin-like epidermis is induced only by the presence of an underlying dermis, i.e. only on the fetal body. Naaktgeboren & Zwillenberg (1961) suggest that there is probably a humoral agent responsible for the differentiation of the epidermis, as they found amniotic pustules only in the presence of a well vascularized amnion. Our findings seem to support this suggestion. The main point is not whether there is a dermis or another type of connective tissue, but whether it is well vascularized or not.

The amniotic pearls on the umbilical cord (fig. 5) had either a short or a long stem. In histological sections they appeared to consist mainly of connective tissue. A cornuated and multilayered amniotic epithelium was not present. These pearls greatly resemble the amniotic pustules of the horse, described by Naaktgeboren & Zwillenberg (1961) as "Amnionpilze", although they contained no pigment, as they do in the horse .

3. Placental girdle

According to Mossman (1957) the Aardvark has an endotheliochorial placenta. Cross sections of the placenta, perpendicular to the direction of the villous ridges (fig. 2, square 1), showed broad-based primary villi, which branched into many secondary and small tertiary villi (fig. 9). The vascularization even of the largest villi was very indistinct. The chorionic epithelium was still present almost everywhere. It consisted of cuboidal, or more frequently of flattened cells, which showed no clear cell-boundaries (fig. 10). This epithelial layer was indented by capillaries, especially in the tertiary villi, but epithelial cytoplasm always covered the walls of the capillaries.

The dark spots on the placental surface (fig. 2, square 2) consisted of almost bare patches of chorionic membrane, and were encircled by darkly coloured villi. In histological preparations of such a dark spot, the chorionic membrane showed only very small primary villi and was even avillous at many places. The encircling villi were broad-based and branched only into thick secondary villi (fig. 11). The epithelial lining of the avillous chorionic membrane and these large villi consisted of high cuboidal, or sometimes even cylindrical cells (figs. 12, 13). These cells were richly vacuolized and sometimes binucleate. Many cells contained pycnotic nuclei. The vascularization was very distinct in these parts of the placenta and very large

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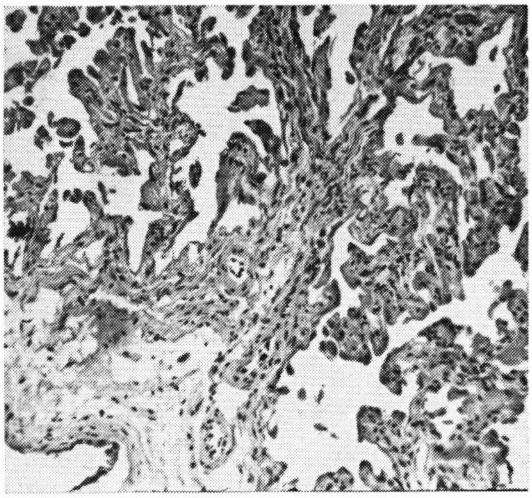

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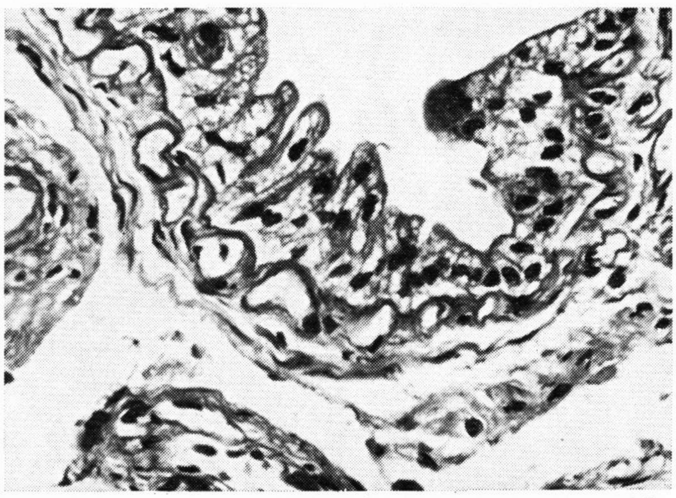
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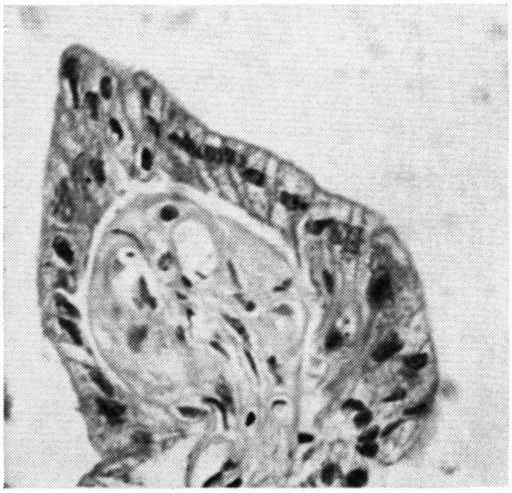
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Fig. 9. Cross-section of the villous part of the placenta (cf. fig. 2, square 1), showing a broad-based primary villus which branches into many secondary and tertiary villi. The allantois is seen at lower left (c. 100x, H.E. stained).
Fig. 10. Cross-section of tertiary villi from the same section as in figure 9. Note the flattened chorionic epithelial cells (c. 425x, H.E. stained).
Fig. 11. Cross-section of a dark spot of the placenta (cf. fig. 2, square 2) at the place where only some small primary villi occur on the chorionic membrane. Some large allantoic blood vessels can be seen in the chorionic connective tissue. The allantoic space is at lower left. Note the very distinct epithelial lining of the villi and chorionic membrane and the many cross-sectioned capillaries immediately beneath the epithelium. (c. 100x, P.A.S. stained).
Fig. 12. Detail of the cross-sectioned chorionic membrane of a dark spot of the placenta (square from fig. 11). The epithelium consists of high cuboidal and cylindrical cells which are richly vacuolized. Many capillaries can be seen beneath this epilthelium (c. 425x, P.A.S. stained).
Fig. 13. Detail of the top of a villus at the border of a dark spot, showing the highly cuboidal epithelial cells, which show very distinct cell boundaries (c. 425x, iron-haematoxylin stained).

capillaries lay close together immediately beneath the chorionic epithelium. These brown spots of the placenta are most likely the foetal remnants of the so-called chorionic vesicles, which Mossman (1957) describes for the intact placenta, although the difference in size between the spots described in this paper (up to 15 mm in diameter) and the chorionic vesicles mentioned by Mossman (1 to 4 mm in diameter) is considerable. This may partly be explained by the difference in age of the placentae studied (nearly full-term and delivered) and by the more vesicle-shaped and vaulted appearance of these parts of the chorionic membrane in utero. Histologically there is a resemblance to the description which Amoroso et al. (1958) gave of the chorionic vesicles of the Hippopotamus and with those of *Lepilemur,* described by Hill (1922).

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