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MORPHOLOGY OF THE BOVINE PLACENTA AT NORMAL DELIVERY

By

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The delivery of the foetal membranes after partus is a physiological process that involves different alterations. Two main factors are associated with it: the myometrial activity and the loosening of the foetal-maternal adherence. *Hallman* (7) regarded the muscular action as essential. He stated that the normal delivery depends on uterine contractions by which the large villi are forced out of the crypts. On the other hand *Williams* et al. (22) assumed that the afterbirth leaves the uterus passively.

Though the uterine motility may be said to play a compulsory rôle, the relationship between the maternal and foetal tissue elements seems to be the determining factor. The firmness with which the chorionic villi are fixed at the caruncular crypts is believed by many authors to be influenced by the turgor caused by the blood pressure. *Pomayer* (15) considered that after partus the caruncles become empty of blood on account of uterine contraction, and thus the foetal placenta could be expelled. *Stewart* (18) expressed the opinion that when the blood leaves the umbilical cord, the vessels of the villi will collapse and a loosening occur. Similar points of view have been forwarded by other authors (6, 16). *Kingman* (9) stated bleeding to be of importance for the loosening of the foetal-maternal adherence. The appearance of defects in the foetal component has also been reported as a cause of delivery. Thus *Kugeler* (10) considered the delivery

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to depend on demarcation of the chorionic villi and *Kennedy* (8) found degeneration or necrosis of the villi to precede normal delivery.

The above mentioned observations and theories are very incomplete, as they are mainly by-products of studies on pathological cases (retentio secundinarum). In order to study the placental morphology at partus and normal delivery we have undertaken an investigation of healthy specimens with the aid of histological and electron microscopical methods. In a following paper we will give a report on the placental morphology in cases of retentio secundinarum.

MATERIAL AND METHODS

Seven apparently healthy cows were examined in connection with partus. After partus the cows expelled their foetal membranes spontaneously after ½ to 3 hours.

Whole placentomes were taken out and cut into small blocks for prompt fixation. After spontaneous loss of the foetal membranes samples were also taken from caruncles and from the expelled foetal cotyledones. After delivery caruncles were taken out at intervals. Thus puerperal caruncles taken after 1, 2, 4, and 8 hours and after 1, 2, and 8 days were examined. The latter material comprised 2 cows only. As the incision might have caused infections the uteri were in the latter cases treated with uteritories containing antibiotics. No metritis occurred. The fixatives used were formaldehyde solution (10 %), Bouin's, Serra's, and Helly's fluides. After fixation in Helly's fluid the tissue blocks were postchromated for 4 days in a saturated solution of potassiumdichromate at 37° C. After dehydration, embedding in paraffine, and sectioning, the staining was performed with hemalum and eosine, Azan, and periodic acid Schiff (PAS). The Serra fixed sections were stretched on etanol and provided with a film of collodion before staining according to the PAS-method was undertaken. Controls were treated with diastase (Zymola) at 37° C for one hour before staining. Frozen sections from formaldehyde fixed blocks were stained with Sudan III or Sudan Black B. The Turnbull blue method for iron (14) and *Washburn's* (19) method for hemoglobin were also performed.

Very small pieces were fixed in buffered isotonic osmium tetroxide solution (12) for 2 hours, dehydrated, embedded in n-butylmethacrylate + n-methylmethacrylate (20:1) polymerized

at 60° C, and cut on a Porter-Blum microtome. The sections were picked up on formvar coated copper grids and examined in a Siemens Elmiskop I at 80 kV.

OBSERVATIONS

The Placentome.

The pre-partum placentome has been described by many authors (*cf.* 1). It is composed of a foetal and a maternal part, which are fused to constitute an organ of physiological exchange between mother and offspring. Branched chorionic villi fit into crypts of enlarged uterine caruncles. The villi consist of vascularized mesencyme provided with a simple layer of a cellular trophoblastic ectoderma. The trophoblast consists of "ordinary" trophoblastic cells and binucleate giant cells. The caruncular crypts are lined with a simple cuboidal epithelium of maternal origin. On the cellular level the apposition of the foetal membranes to the uterine mucosa is represented by adherence of trophoblast to cryptal epithelium. The trophoblastic and cryptal
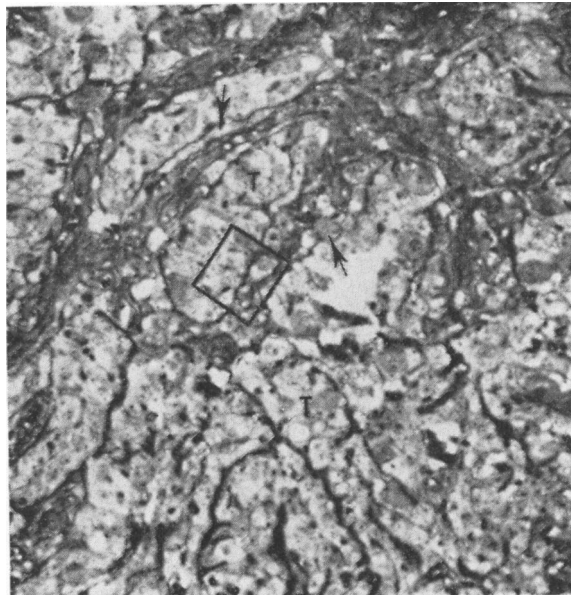

F i g. 1. Chorionic villi and caruncular crypts from placentome taken between partus and normal delivery of the foetal membranes. Note the distinct line of junction between the trophoblast (T) and the cryptal epithelium (arrows) and between trophoblastic epithelia of adjacent twigs of chorionic villi. Azan. Photomicrograph. 200 ×.

cells are provided with microvilli, which interdigitate with each other, thus creating a considerably increased contact surface between the foetal and maternal tissues (2).

There are some differences between the structure of post-partum placentomes and that of placentomes at earlier stages of gestation. In some crypts the lining maternal epithelium is more
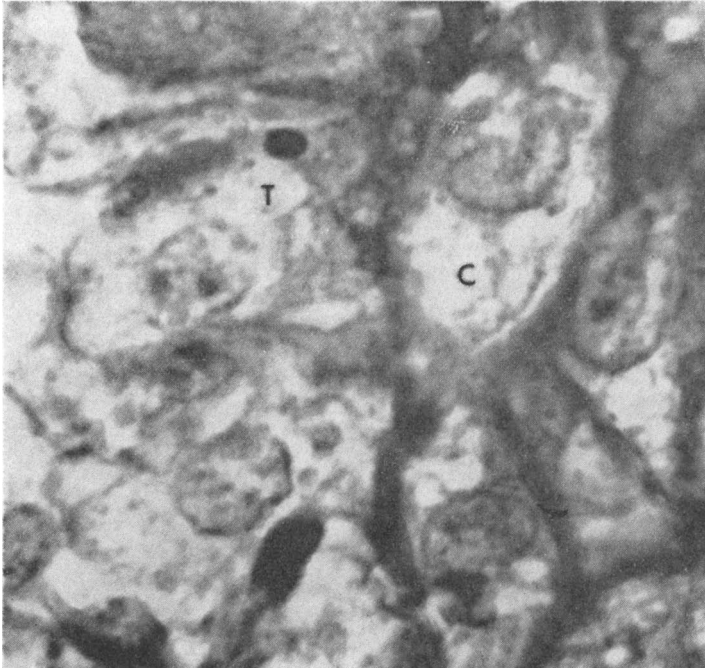

F i g. 2. Enlargement of inset from Fig. 1. showing the line of adherence between trophoblast (T) and cryptal epithelium (C). 200 ×.

or less atrophied and flattened. In a few crypts it is absent leaving the cryptal basement membrane denudated. On the other hand plasmodia with 10—20 nuclei occur in part of the cryptal epithelium. The mentioned phenomena are also found in mature placentomes pre-partum (1).

At earlier stages of pregnancy the microvilli of the trophoblastic and cryptal epithelial cells are not seen in the light microscope. In post-partum placentomes and in mature pre-partum placentomes the trophoblastic microvilli can be rendered visible. Thus after fixations causing shrinkage (e. g. Bouin's fluid) artificial separations between trophoblast and cryptal epithelium
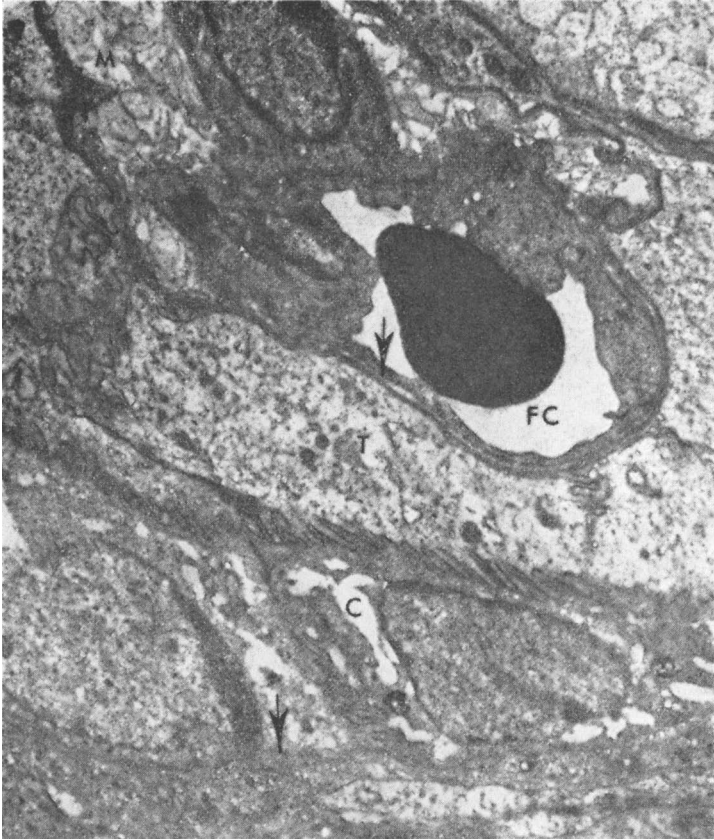
F i g. 3. Detail of placentome showing the apposition between a cho-
rionic villus (top) and a caruncular crypt (bottom). T = trophoblast,
M = mesenchyme, FC = Foetal capilary (containing a red cell), C =
cryptal epithelium. Arrows indicate foetal (middle) and maternal
(bottom) basement membranes. Electronmicrograph. 8300 ×.

occur. In such areas of the sections a distinct brush border is seen
at the surface of the trophoblast. The border is more stainable
the rest of the cytoplasm. In the cryptal epithelium the brush
border is difficult to see in the light microscope.

In large areas of post-partum placentomes the line of junction
between trophoblast and cryptal epithelium is highly stainable
(Figs. 1 and 2). The stained suture is revealed if the cells are pre-
served in close apposition. This is effected after fixation, e. g. in
Helly's fluid. The stainability is very conspicuous after staining
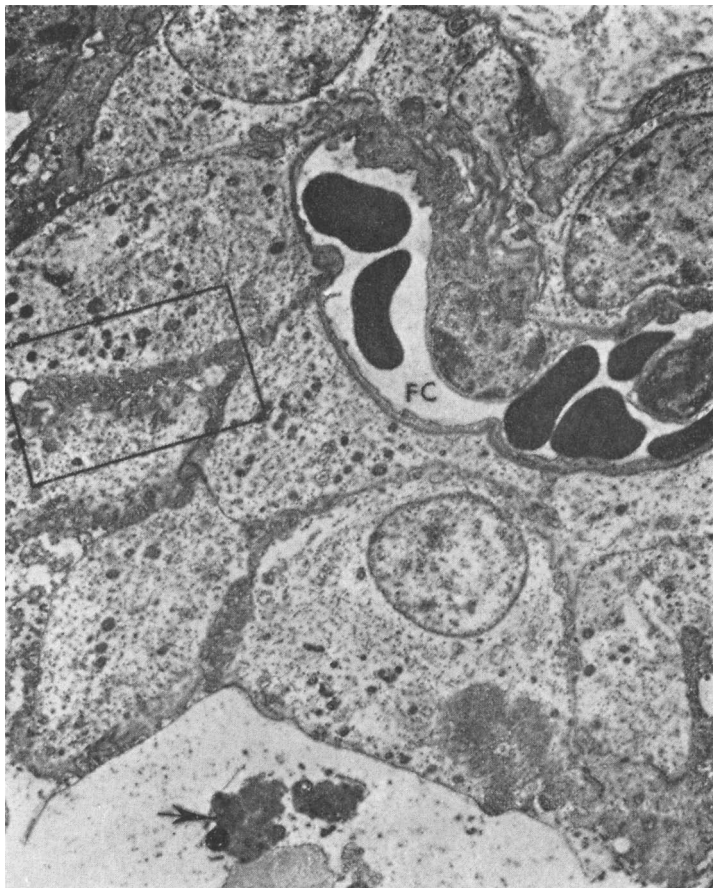with Azan, which gives an intense bluish red colour. The line is

F i g. 4. Trophoblastic cells with microvilli. The light cell (bottom) contains pigment (arrow). FC =-foetal capillary. Electronmicrograph. 4300 ×.

also **PAS**-positive resisting treatment with diastase. Not all the twigs of a primary chorionic villus fit into corresponding crypts. When trophoblast from one twig adheres to that of another the line of contact is stained as described above. Thus the stainability is (mainly) centered in the trophoblastic brush border (Fig. 1).

The stainable line is represented on the ultrastructural level respectively by interdigitating microvilli (Fig. 3) and by trophoblastic microvilli only (Fig. 4 and 5). The foetal maternal suture and especially the trophoblastic microvilli are more conspicuous than at earlier stages of gestation as described by *Björkman* and *Bloom* (2). The trophoblastic cell membranes also show an in-
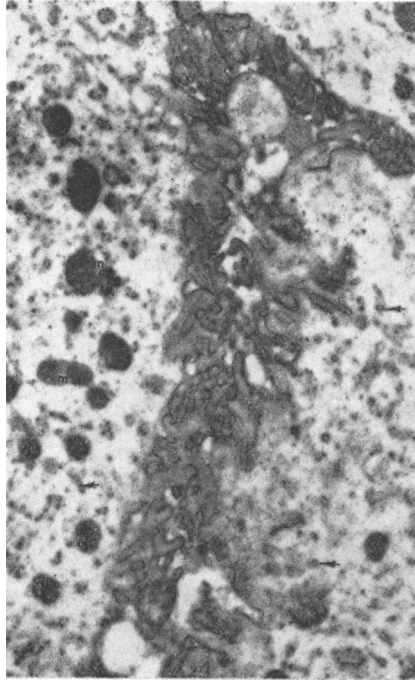
F i g. 5. Enlarged inset of Fig. 4. m = mitochondria. Arrows indicate tubules of the endoplasmic reticulum. Electronmicrograph. 23000 ×.

creased area, following sometimes a very tortuous course (Fig. 8). The cryptal cells, which are variable in shape (low columnar to squamous) contain great amounts of sudanophilic droplets. In electron micrographs the latter are seen as round osmiophilic droplets. In placentae from earlier stages of gestation they are surrounded by concentric lamellae and occur at different stages of development (2). At term the lamellae are diffuser and the lipid droplets appear as dense granules within vacuoles. Exceptionally, less dense droplets are observed (Fig. 6).

In mature placentomes part of the trophoblast contains phagocytized erythrocytes and inclusions, which are moderately PAS-positive and resists treatment with diastase. The inclusions occur as a rule solitary in the villi of secondary or higher order. In the periphery of the placentome and among the primary villi the trophoblast contains large conglomerates, which are also associated with clusters of a brown pigment (Fig. 7). Staining with Turnbull blue reveals small granules containing iron, which are associated with the pigment. However, most of the latter re-
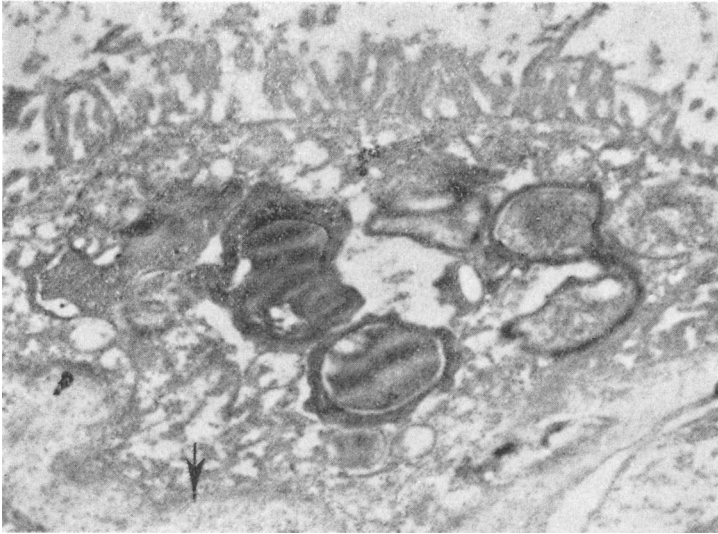
F i g. 6. Epithelial cell from a crypt where the chorionic villus has been obliterated though the afterbirth has not yet been delivered. Note intact microvilli and lipid droplets of varying density. Arrow indicates basement membrane. Electronmicrograph. 11000 ×.
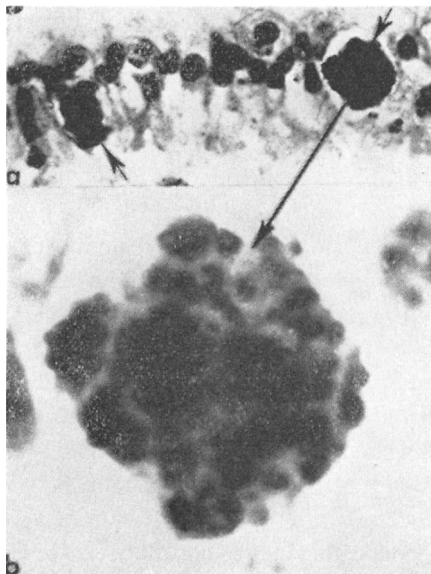

F i g. 7. a) Trophoblast containing hemoglobinogenous pigment (arrows). Azan. Photomicrograph. 400 ×. — b) Right pigment cluster enlarged to 1800 × (bottom).
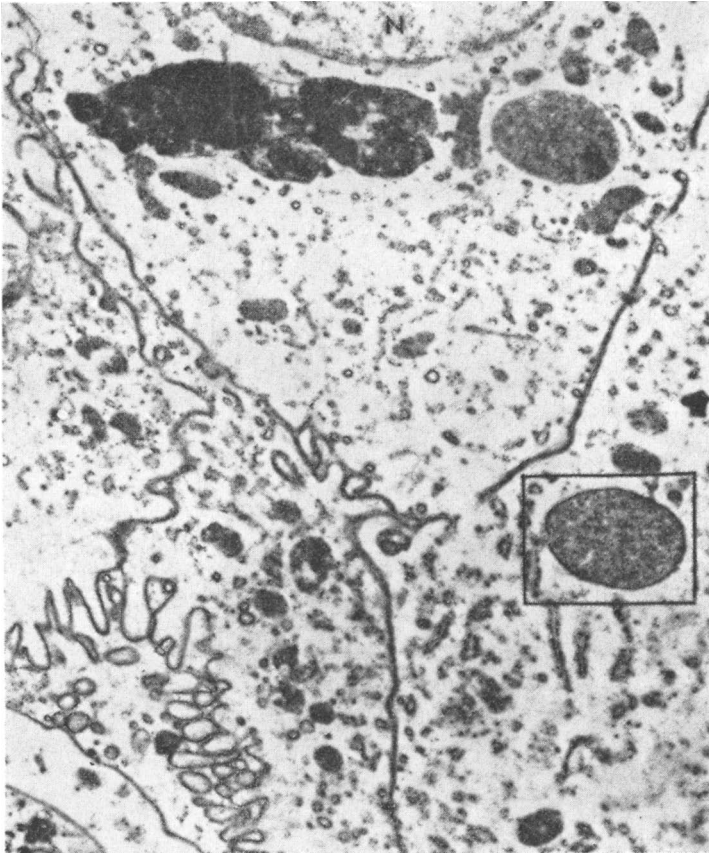
Fig. 8. Trophoblast with inclusions probably containing iron. N = nucleus. Note the tubules of the endoplasmic reticulum and the partly tortuous course of the cell membranes. Electronmicrograph. 12000 ×.

mains unstained. Though positive reaction for hemoglobin is found in the erythrocytes, the pigment is not stained with this method. In electron micrographs the small inclusions appear as ovoid bodies with an internal granular structure of high density. They are sometimes provided with a limiting membrane, but in other cases no membrane is visible (Figs. 4, 8, and 9). The coalescued bodies form very irregular masses containing small rounded bodies of still higher electron density. The trophoblast also contains a moderate amount of glycogen.

In most parts of the placentome the chorionic villi are intact. In some parts, however, the foetal tissue is obliterated and the crypts are more or less filled with detritus. In such cases the

F i g. 9. Enlarged inset of Fig. 8. 80000 ×.

cryptal epithelium is often intact, but it may be lost to some extent or rather completely absent. In such crypts maternal epithelium with intact microvilli has been observed (Fig. 6).

The caruncles after loss of the foetal membranes.

Immediately after the foetal membranes have been expelled the uterine caruncle remains as a framework of septa consisting of connective tissue. Most of the crypts are empty and have collapsed to some extent. As a rule they are still lined with a cuboidal epithelium (Fig. 10). It varies in appearance and is fairly high in some crypts. In other crypts it is lower or rather squamous. There are also crypts with completely denuded walls. The epithelial cells contain sudanophilic lipid droplets with a somewhat uneven distribution. In sites where the chorionic villi have degenerated before the delivery of the afterbirth the crypts contain detritus of foetal origin. Not all crypts have been emptied. Thus remnants of chorionic villi are retained in various parts of the placentome. They are still in intimate contact with the maternal tissue. In these cases the foetal-maternal line of junction is not so highly stainable as usual.

During the first hours after the delivery no remarkable alter-

ations in the picture are observed, but then gradually the cryptal epithelium is lost. Most of the cells seem to be loosely attached to the basement membrane and have a tendency to fall off. They are better preserved in situ when Helly's fixative is used than after fixation e. g. with Bouin's fluid. The further course of the puerperal changes in the caruncle is subject to variation in different specimens, but the main features are involution of the crypts and proliferation of fibroblasts.
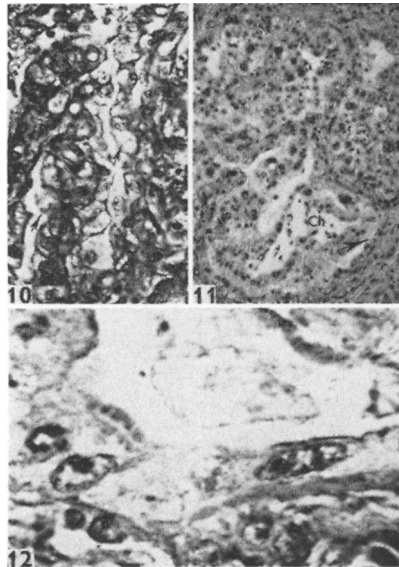

F i g. 10. Caruncular tissue 5 hours post partum and 4 hours after delivery of the afterbirth. Note the collapsed crypts and the remaining cryptal epithelial cells (arrows). Azan. Photomicrograph. 500 ×.

F i g. 11. Carucle one day after delivery of the afterbirth. Retained chorionic villi in contact with cryptal epithelium are still intact. Ch = chorionic villus. Arrow indicates line of foetal-maternal contact. Hemalum + eosin. Photomicrograph. 175 ×.

F i g. 12. Caruncular crypt with intact epithelium 8 days after delivery of the afterbirth. Note the brush border Azan. Photomicrograph. 1750 ×.

After one day the epithelium has been lost in many crypts whereas it is present in others. Then, in a cryptal complex belonging to one (expelled) primary villus the small cryptal units show the same picture. In other regions of the placentome other stages of desquamation and/or degeneration of the cryptal epi-

thelium is seem. In af few small crypts the epithelium has proliferated and large cells fill up most of them. Most of the retained chorionic villi have become necrotic but also surviving villi are seen where the trophoblast adheres to vital cryptal epithelium (Fig. 11). The septa have increased in thickness and contain growing fibroblasts.

After 2 days most of the cryptal epithelium has disappeared and most retained villi are degenerated. Lipid droplets are found in the few remaining cryptal cells and also in the degenerated trophoblast. In the latter case the lipid has become unmasked as it is not seen in intact trophoblast.

Though most of the cryptal epithelium is lost after 2 days, some crypts still contain intact epithelium after 8 days. At this stage the brush border of the cryptal cells has become clearly visible in the light microscope (Fig. 12). Some islets formed by retained chorionic villi are still seen. They are necrotic or partly intact.

The expelled afterbirth.

In specimens taken immediately after spontaneous delivery of the foetal membranes the chorionic villi have a morphology similar to that before expulsion. The villi are to a large extent provided with intact trophoblastic cells. The brush border is
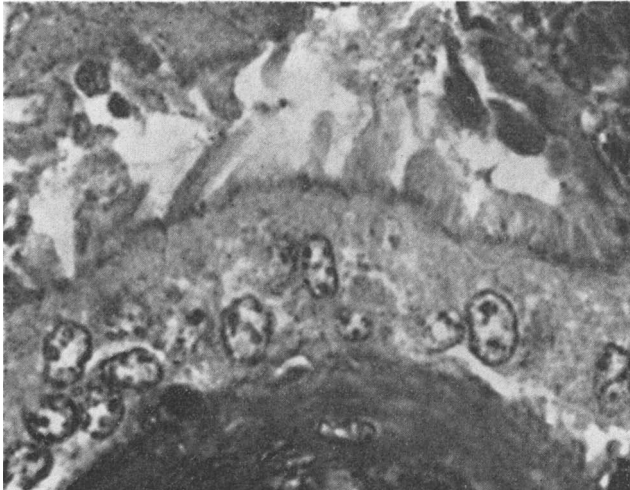

F i g. 13. Trophoblast lining a primary villus of expelled afterbirth. Note the brush border. Azan. Photomicrograph. 600 ×.

often conspicuous and shows higher stainability than the rest of the cytoplasm (Fig. 13). The trophoblast is best preserved along the coarse primary villi, but also twigs of the branched villi may be intact. On the other hand some villi are disturbed. Some of the tissue is obliterated before expulsion as shown above. Other villi have been mechanically damaged during expulsion. No maternal tissue has been observed in the expelled afterbirth.

DISCUSSION

After partus the afterbirth should be delivered within a few hours. The time of normal delivery is generally stated to be within 6 hours after partus. Also longer time lapses are considered by some authors to be normal: *Boyd* (3) 10 hours, *Götze* (6) 12 hours, *Moore* (11) 24 hours. In our material the time varied between ½—3 hours and the material was thus normal in that respect.

The manner in which the placental tissue is anchored at the endometrium is only hinted at in the literature. The chorionic villi are considered by some authors (6, 15, 16, 18) to be fixed at the crypts predominantly by the influence of the blood pressure. However, it seems strange that the effect of dimished blood pressure should manifest itself as late as hours after the umbilical cord has been torn off. Other factors influencing the maintenance of foetal-maternal contact are probable. *Williams* (21) considered "physiological magnetism" to be of importance.

As has been pointed out above the trophoblast adheres to cryptal epithelium. As the villi are branched, the area of contact is considerable. Furthermore it is immensely increased by the mutual interdigitation of microvilli. It may be assumed that the firmness with which the cells adhere to each other determines the keeping of the foetal placenta *in situ*. The cellular adhesiveness may partly depend on the molecular constellation between the surfaces (20). Though the factors are not entirely known, it seems unlikely that a disjunction brought about by purely mechanical forces would not damage the tissue to a considerable extent.

In our material, we have found retained chorionic villi, which must have been torn off, though not necessarily as a consequence of demarcation as stated by *Kugeler* (10). We have also found obliteration of foetal tissue within the crypts as has been described by *Kennedy* (8). These phenomena occur normally, but

they affect only minor parts of the placentomes and thus seem to play a subordinate rôle in the loosening process.

In most of the maternal as well as the foetal parts of the placenta the epithelia· remain unaffected after separation. Thus the natural site of separation is at the line of apposition between the trophoblast and the cryptal epithelium. Particular attention may be called to the stainability of this suture. After partus it is intensely stainable. In parts where chorionic villi have been retained the stainability of the line is somewhat lower. It may therefore be inferred that the cell surface is changed by the attack of certain enzymes (5) or that a stainable substance is elaborated at the trophoblastic surface, probably as a result of enzymatic activity (17). This might influence the intensity of surface interactions and faciliate the segregation of the foetal and maternal epithelia from each others.

Though not implicated in the loosening process the inclusions in the trophoblastic cells are of interest from other points of view. Glycogen has not been found in the trophoblast of ealier placentomes (1). In the mature placentome the trophoblast contains a moderate amount of glycogen. This storage may be interpreted as a sign of diminished activity of the trophoblast.

The trophoblastic cells contain a brown pigment derived from phagocytized erythrocytes. No hemoglobin has been found in the pigment, but associated with the clusters small bodies containing iron have been observed. The bodies appear polymorphic and dense in the electron microscope. Some of them have a structure similar to the "round bodies" demonstrated by *Palade* (13) in macrophages. He considered them to represent terminal appearance of phagocytic vacuoles and the dense granular material they contain to be a metal-organic compound. Thus when the red cells have been phagocytized it seems as if the hemoglobin delivers its iron, which is then eliminated from the trophoblast, presumably to be resorbed by the foetus. The ultrastructure of the small bodies and their above discussed significance is in accordance with the concept of lysosomes as presented by *de-Duve* (4).

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SUMMARY

A morphological investigation of the normal placenta in cattle in
connection with partus has been undertaken with the use of histologi-
cal, histochemical, and electron microscopical methods. The pla-
centomes have been studied between partus and the spontaneous de-
livery of the afterbirth. Furthermore, the caruncles and the expelled
foetal cotyledones have been investigated after delivery. Before deli-
very most of the trophoblast is in contact with the cryptal epithelium.
The border between the two kinds of cells is very stainable. To a large
extent it consists, on the ultrastructural level, of interdigitating ma-
ternal and foetal microvilli. Areas with partly obliterated chorionic
villi as well as parts with atrophied cryptal epithelium are present.
Chorionic villi are loosened from the crypts along the contact surface
between the trophoblastic and cryptal epithelium. After spontaneous
delivery of the afterbirth the cryptal epithelium as well as the tropho-
blast are mostly intact. The significance of the border of contact in con-
nection with the loosening of the cells from each other is discussed.

The trophoblastic cells phagocytize erythrocytes. After the iron
has been resorbed there remains a brown pigment in the cells.

ZUSAMMENFASSUNG

Morphologische Untersuchung der normale Rindeplazenta.

Eine morprologische Untersuchung über die normale Rindeplazenta im Anschluss zu Partus ist ausgeführt worden. Histologische, histochemische und elektronenmikroskopische Methoden sind benutzt worden. Die Plazentomen wurden zwischen Partus und dem spontanen Abgang des Nachgeburts studiert. Die Karunkeln und die ausgestossenen fötalen Kotyledonen wurden nach dem Abgang untersucht. Vor dem Abgang ist das Trofoblast zum grössten Teil mit dem Kryptenepithel in Kontakt. Die Grenzlinie ist sehr farbbar. Auf ultrastructurellem Niveau besteht die Linie aus mütterlichen und fötalen Mikrozotten, die unter einander hineingehen. Es kommen Gebiete vor wo die Chorionzotten aufgelöst worden sind. Es kommt auch atrophisches Kryptenepithel vor. Die Chorionzotten machen sich von den krypten längs der kontaktfläche zwischen Trofoblast und Kryptenepithel frei. Nach dem spontanen Abgang der Nachgeburt ist das Trofoblast wie auch das Kryptenepithel zum grössten Teil unbeschädigt. Die Bedeutung der Kontaktfläche für die normale Zusammenhangstrennung zwischen den Zellen wurde diskutiert.

Die Trofoblastzellen phagozytieren rote Blutkörperchen. Nachdem das Eisen resorbiert worden ist, bleibt ein braunes Pigment in den Zellen übrig.

SAMMENFATTNING

Morfologisk undersökning av nötkreaturens normala placenta.

En morfologisk undersökning med användande av histologiska, histokemiska och elektronmikroskopiska metoder har utförts i anslutning till förlossningen på nötkreaturens normala placenta. Placentomen ha studerats mellan partus och det spontan avlossandet av efterbörden. Vidare ha karunklerna och de utstötta fötala kotyledonerna undersökts efter avgången. Före avgången är större delen av trofoblastet i kontakt med kryptepitelet. Gränslinjen mellan de båda slagen av celler är starkt färgbar. Den utgöres på ultrastrukturell nivå av materna och fötala mikrovilli, som gå in mellan varandra. Områden med delvis upplösta chorionvilli förekomma också liksom partier med atrofierat kryptepitel. Chorionvilli lösas från kryptorna utefter kontaktytan mellan trofoblast och kryptepitel. Efter spontanavgång av efterbörden visar sig såväl kryptepitelet som trofoblastet till större delen vara oskadat. Gränsytans betydelse för normalt lösgörande av cellerna från varandra diskuteras.

Trophoblastcellerna fagocytera erythrocyter. Sedan järnet resorberats återstår ett brunt pigment i cellerna.