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Ultrastructure of the mesenchymal layers of the human chorion laeve

A. D. HOYES

Department of Anatomy, St Mary's Hospital Medical School, London, W.2

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INTRODUCTION

The mesenchyme lining the inner surface of the human chorion arises, in common with the mesenchyme associated with the other extra-embryonic membranes, from the reticular network of extra-embryonic mesoderm which appears within the cavity of the blastocyst immediately after implantation. This tissue resembles that of both the yolk sac and body stalk in being the site of development of an extensive system of vascular channels; through anastomosis with the vessels formed in the body stalk, these soon become an integral part of the fetal circulation. Further development of the vessels is largely confined to the placental region of the chorion, and the transformation of the rest of this structure into the chorion laeve is accompanied by the early disappearance of the circulation from the underlying mesenchyme. However, Lister (1968) has recently described vascular structures in the mesenchyme at term and, although it is probable that the cells which contribute to the formation of the vessel walls possess capacities for functional activity which differ from those of the other components of this tissue, the extent to which such cells persist in the mesenchyme after the disappearance of the circulation is unknown. Further evidence relating to the fate of the blood vessels and to the morphology of the various types of cell present in these layers of tissue has therefore been sought in an ultrastructural study of material obtained between the 2nd and 7th months of pregnancy and at term.

MATERIALS AND METHODS

Specimens of chorion laeve were obtained from 6 placentae following normal delivery at term and from a series of 28 conceptuses delivered by abdominal hysterotomy or hysterectomy. The crown–rump length of the fetuses in these specimens varied between 14 and 210 mm and, since a reliable menstrual history was rarely available, this figure was used to classify the specimens into the same groups as those used by Boyd & Hamilton (1970) in their recent study of the placenta. The specimens were fixed for 2 h in veronal-buffered osmium tetroxide (Palade, 1952) or for 30 min in 2 % glutaraldehyde in cacodylate buffer (Gordon, Miller & Bensch, 1963), the latter then being washed in buffered sucrose and postosmicated for 1 h in Palade's (1952) fixative. Blocks from each of the specimens were dehydrated in ethyl alcohol and embedded in Araldite according to the schedule given by Glauert (1961).

For light microscopical investigation, thick sections of Araldite-embedded material

were cut on a Porter–Blum ultramicrotome, mounted on glass slides and stained with toluidine blue. Ultrastructural studies were undertaken on thin sections mounted on uncoated grids and stained with lead (Karnovsky, 1961) or with both lead and uranyl acetate. The sections were then examined and photographed in a Siemens Elmiskop 1 or Philips EM 300 electron microscope.

RESULTS

Light microscopy

Small vessels containing fetal blood cells were seen in thick sections of material from all three specimens obtained during the 2nd lunar month, as well as in material from conceptuses with fetuses of 41 and 54 mm crown–rump length. While vessels were not apparent in other material obtained during the 3rd month, structures resembling vascular channels were seen in specimens of up to 6 months in age. However, blood cells could not be identified in the lumen of these structures, their lining cells were irregular in shape, and there appeared to be defects in their walls.

Mesenchymal cells were particularly numerous in the youngest specimens, but much less so in the later stages of gestation. In all specimens the cells formed elongated processes usually disposed in planes parallel to that of the chorionic epithelium. In the youngest material the cell bodies were generally irregular in shape, but in the older specimens and at term they were often elongated or rounded. In these specimens lipid droplets were also often present in the cytoplasm, especially in areas in which they were numerous in the overlying epithelium and decidua. Cells containing the cytoplasmic vacuoles characteristic of Hofbauer cells were only occasionally visible in the youngest specimens and at term, but were more common in those obtained during the 5th, 6th and 7th months. In specimens in which the amnion remained attached to the chorion, cells of this type were also numerous in the zone of spongy tissue separating the amnion from the chorion.

Electron microscopy

In addition to the vessels seen with the light microscope, much of the youngest material contained structures resembling the vascular sprouts formed during the early stages of recanalization of thrombosed vessels (Wiener & Spiro, 1962) and showing little evidence of the development of a lumen. In the definitive vessels the lumen was also sometimes small and devoid of cellular content, but usually it was well developed and contained either nucleated or anucleate red cells. Many vessels seen in two of the specimens obtained during the 3rd month also contained fetal erythrocytes. In the oldest of these specimens, other cells, resembling in their fine structure the megakaryocytes formed in the yolk sac (Hoyes, 1969) and subsequently in the liver (Zamboni, 1965), could be seen in the vessels (Fig. 1). In some instances the lumen was filled with cellular debris.

In the vessels containing cellular debris there was a marked increase in the electron density of occasional endothelial cells; this was similar to that reported in ischaemic endothelial cells in the liver (Steiner, Carruthers & Kalifat, 1962). Otherwise there was no evidence of endothelial degeneration, and, both in the youngest specimens and in those obtained during the 3rd month, the vascular endothelium was usually com-

posed of a continuous layer of apparently viable cells united by tight junctions and by the other types of attachment plaque normally present between endothelial cells. Pinocytotic activity was normally evident at the plasma membranes, and vesicles were numerous in the region of the perinuclear Golgi apparatus. Scattered microtubules were sometimes visible in the cytoplasm; filaments were conspicuous and, especially in vessels containing cellular debris, were frequently concentrated in localized areas of the cytoplasm (Fig. 1). Condensations of filamentous material close to the plasma membrane were often evident in such cells, and masses of cytoplasm, sometimes of a considerable size, frequently projected from their external surface (Fig. 5).

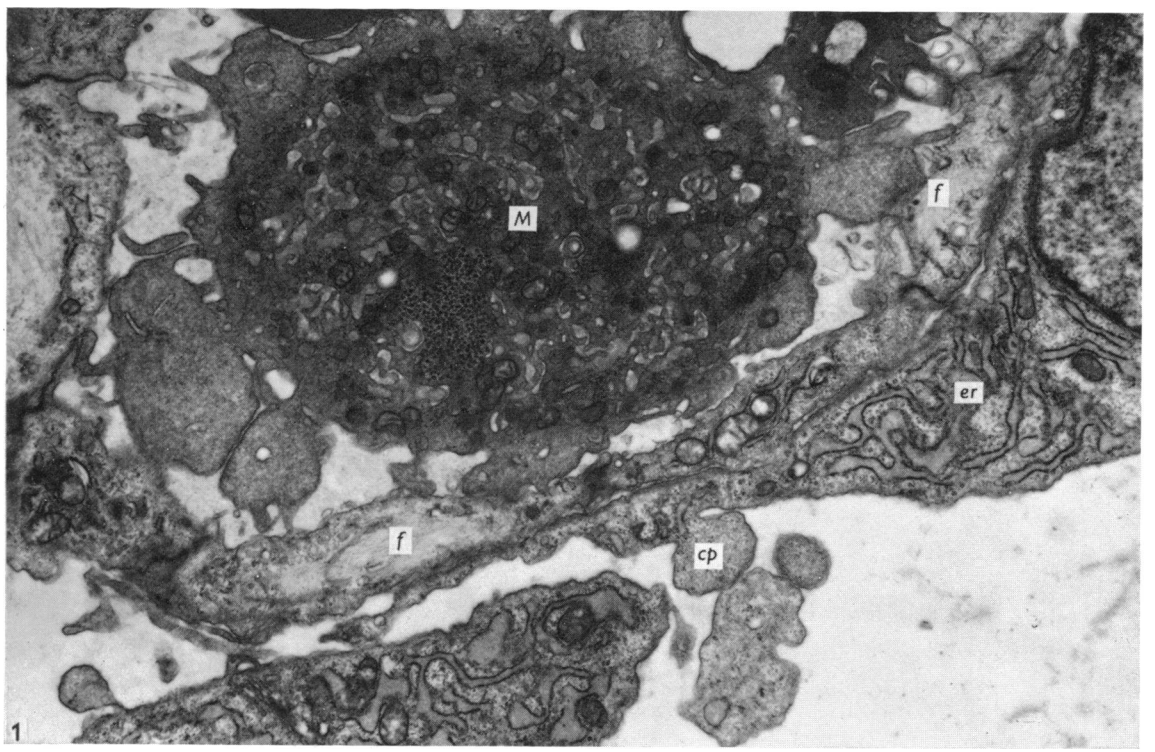
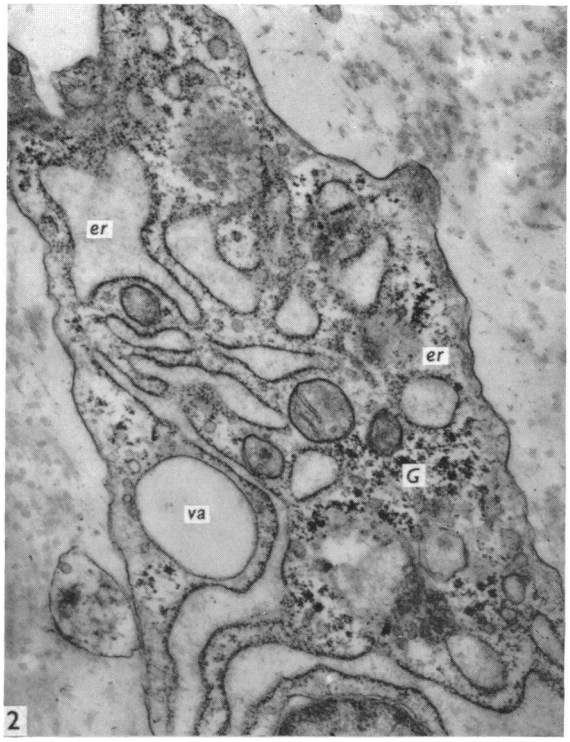


Fig. 1. Megakaryocyte (*M*) in lumen of blood vessel in a specimen obtained during the 3rd month. *f*, cytoplasmic filaments in endothelial cells; *cp*, cytoplasmic projection from periendothelial cell; *er*, granular endoplasmic reticulum. Fetus CR 54 mm. Lead stain. ×14000.

The smaller vessels were lined only by endothelium, but in the larger vessels this was almost entirely covered by the processes of one or more periendothelial cells. These were united to the endothelial cells by occasional attachment plaques, and irregular masses of cytoplasm, similar to those formed from the endothelial cells, commonly projected from their outer surface (Fig. 1). Glycogen was more abundant in the cytoplasm than in the endothelial cells, filaments were few, and the cells con-



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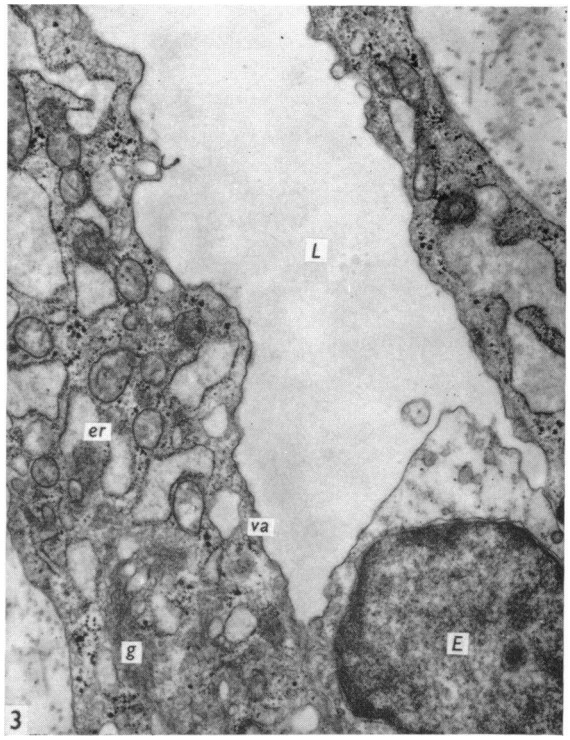

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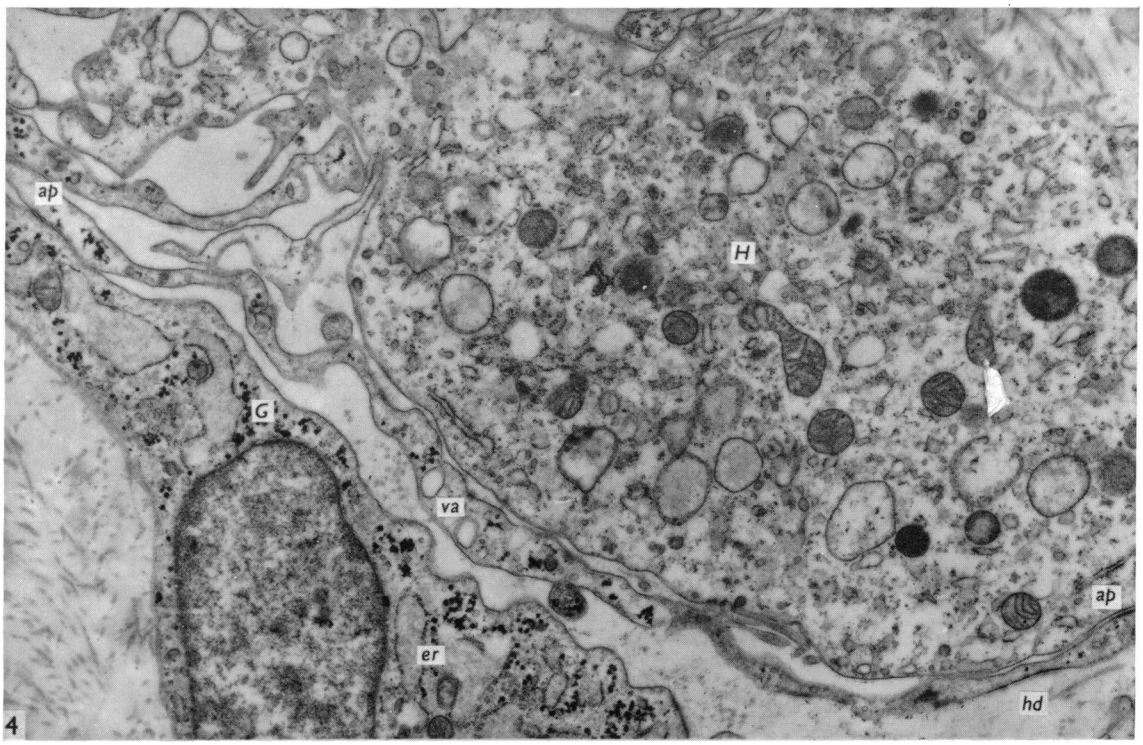

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tained a prominent Golgi apparatus and a highly developed granular endoplasmic reticulum (Fig. 1).

Although the vessel-like structures present in the older specimens sometimes consisted of a single layer of cells surrounding a central, and usually empty, lumen (Fig. 3), the cells were often separated from one another, so forming the sites of open communication between the lumen and the exterior which were seen with the light

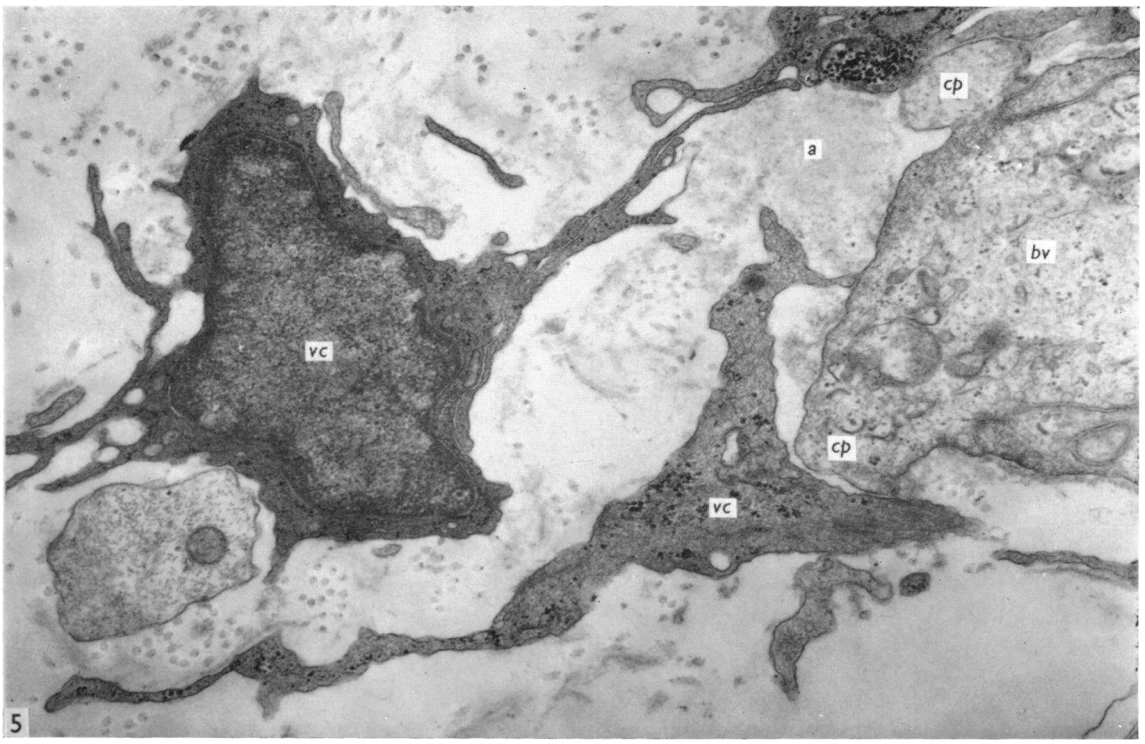


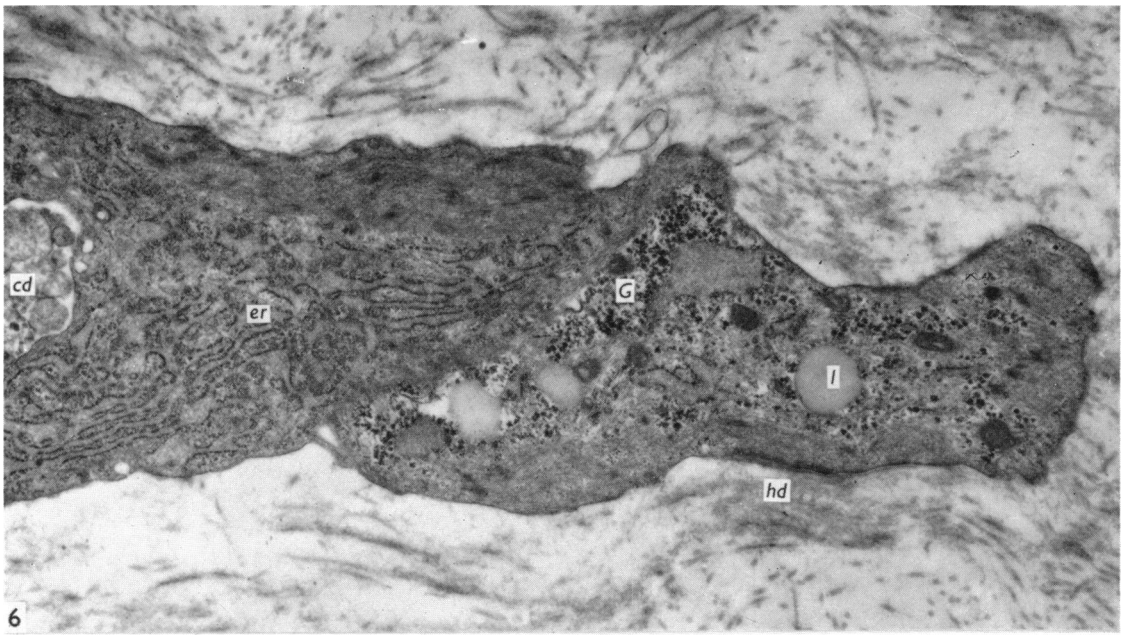
Fig. 5. Elements of the second population of mesenchymal cells (*vc*) close to the endothelium of a blood vessel (*bv*). *cp*, Cytoplasmic projections from endothelial cell; *a*, amorphous material in extracellular space. Fetus CR 54 mm. Lead stain. × 12200.

microscope, and in some instances there was such extensive separation and disorganization of the cells that the lumen was difficult to define (Fig. 4). The various components of these structures were still united by occasional attachment plaques

Fig. 2. Late stage of differentiation of a mesenchymal fibroblast. *er*, granular endoplasmic reticulum; *va*, cytoplasmic vacuole; *G*, glycogen. Fetus CR 116 mm. Lead stain. × 23500.

Fig. 3. Part of vessel-like structure in mesenchyme in 5th month. *L*, lumen; *g*, Golgi apparatus; *er*, granular endoplasmic reticulum; *va*, cytoplasmic vacuole; *E*, cell containing scattered vesicles. Fetus CR 110 mm. Lead stain. × 15500.

Fig. 4. Part of vessel-like structure showing marked disorganization of cells. *ap*, attachment plaques; *er*, granular endoplasmic reticulum; *G*, glycogen; *H*, Hofbauer cell; *hd*, hemidesmosome-like structure on elongated process of cell containing scattered vacuoles (*va*). Fetus CR 128 mm. Lead stain. × 14000.



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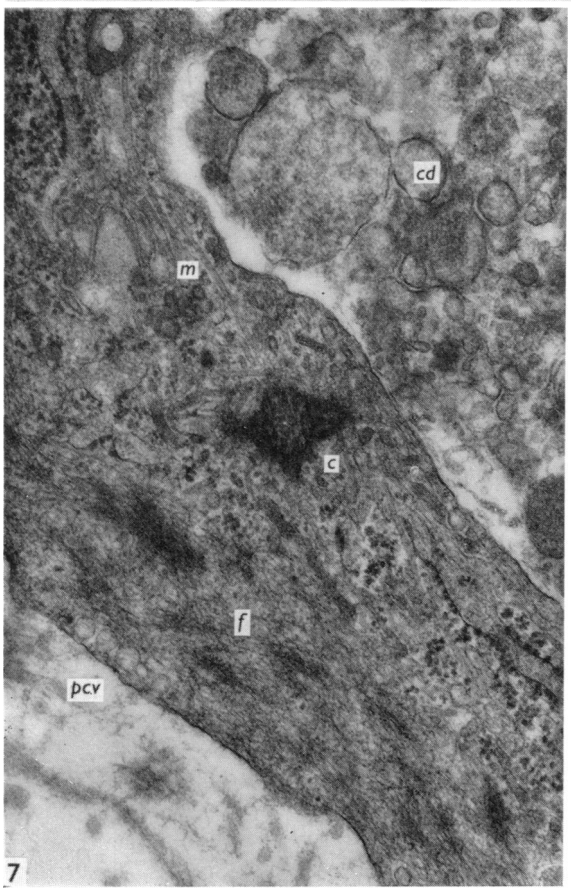

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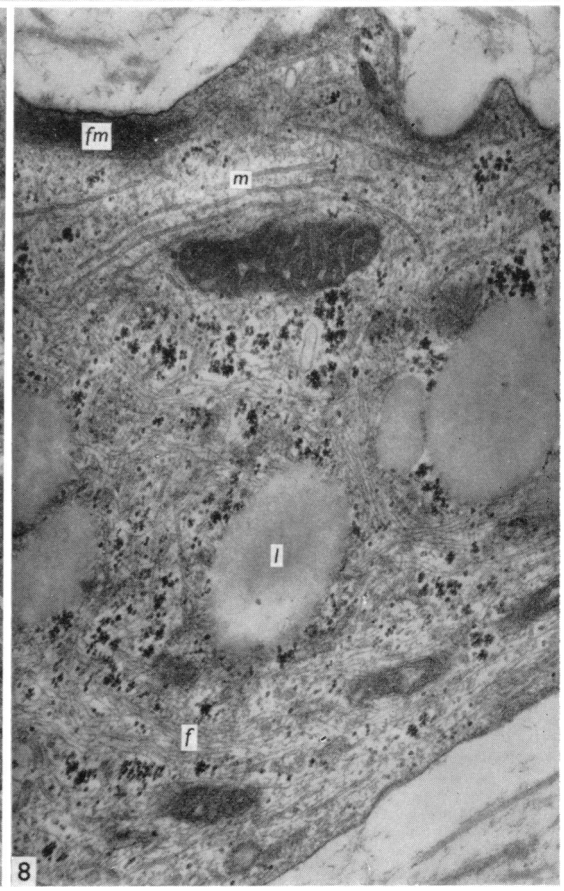

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Fig. 4), but cells resembling those of the endothelium were rare, and most contained a well-developed Golgi apparatus, deposits of glycogen, and numerous sacs of endoplasmic reticulum. These were often dilated and irregular (Figs. 3, 4), and there was evidence both of pinocytotic activity at the plasma membrane and of the formation of membrane-bounded vacuoles in the cytoplasm (Fig. 3). Occasional cells had the typical ultrastructural features of Hofbauer cells (Fig. 4).

Other cells sometimes present in the vessel-like structures had elongated processes and possessed hemidesmosome-like structures on their plasma membranes (Fig. 4). Such cells also showed evidence of at least limited pinocytotic activity but, except for scattered vesicles and small vacuoles (Figs. 3, 4), they contained very few organelles, and filaments were not normally present in their cytoplasm.

In the specimens obtained during the 2nd month many of the extravascular cells were morphologically similar to those identified as undifferentiated mesenchymal cells in the human yolk sac (Hoyes, 1969). However, some showed evidence of differentiation of a similar type to that seen in the cells of the amniotic mesenchyme during the same stage of gestation (Hoyes, 1970) and contained a large Golgi apparatus and numerous sacs of granular endoplasmic reticulum. Small deposits of glycogen, scattered microtubules and occasional groups of fine filaments were also present, and localized areas of increased electron density could sometimes be seen among the filaments. Lipid droplets, on the other hand, were scarce and there was little evidence of pinocytotic activity, or of the development of structures such as hemidesmosomes.

By the end of the 3rd month most cells of this type contained a large Golgi apparatus and a highly developed granular endoplasmic reticulum. The sacs of endoplasmic reticulum were sometimes dilated and irregular, and, in material obtained at somewhat later stages of gestation, fragmented to form structures which were more or less circular in section (Fig. 2). These changes were usually accompanied by a marked reduction in the size of the Golgi apparatus, the formation of membrane-bounded vacuoles in the cytoplasm (Fig. 2) and the appearance of increased pinocytotic activity. Glycogen, although still present (Fig. 2), was generally reduced in amount and the filaments present in the cytoplasm in the younger specimens were now rarely apparent.

Cells with extensive fragmentation of the sacs of endoplasmic reticulum and further development of cytoplasmic vacuoles were not uncommon in specimens of up to 6 months in age, and appeared to represent the final stages in the transformation of cells of this type into Hofbauer cells. These were normally rounded in form and contained a rather featureless and often indented nucleus. As in the amnion (Hoyes, 1970), a Golgi apparatus could not be identified, and the cytoplasm contained numerous vesicles and vacuoles, to the external surface of which ribosomes were

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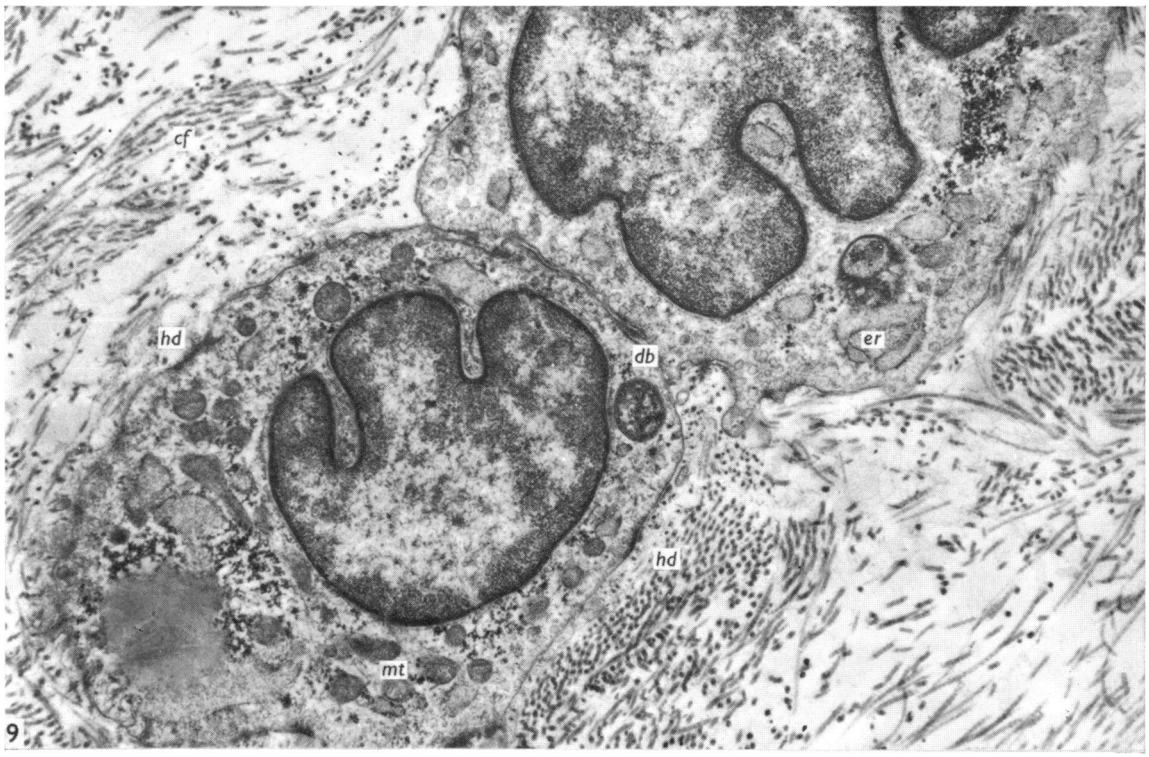
Fig. 6. Process from a type 2 mesenchymal cell. *cd*, cell debris; *hd*, hemidesmosome-like structures; *er*, granular endoplasmic reticulum; *G*, glycogen; *l*, lipid droplet. Fetus CR 54 mm. Lead stain. ×15000.

Fig. 7. Cell debris (*cd*) close to type-2 cell process. *f*, cytoplasmic filaments; *m*, microtubules; *c*,centriole; *pcv*, pinocytotic vesicles. Fetus CR 54 mm. Lead stain. ×44500.

Fig. 8. Type 2 cell process. *f*, cytoplasmic filaments; *fm*, filamentous material close to plasma membrane; *m*, microtubules; *l*, lipid droplet. Fetus CR 54 mm. Lead stain. ×37000.



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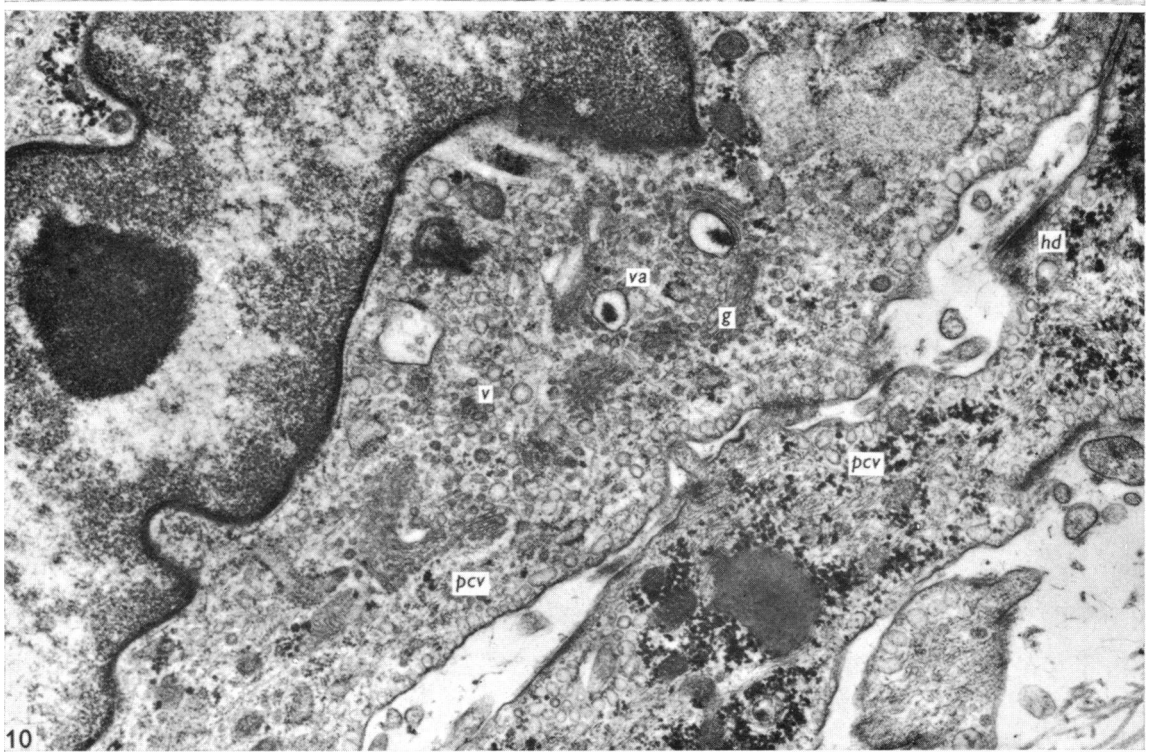

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sometimes adherent. Dense bodies were unusual in the younger specimens, but more numerous in those obtained during the 6th month.

Of the cells present in the mesenchyme at term, very few contained large amounts of granular endoplasmic reticulum, and although cells resembling typical Hofbauer cells could be identified, they differed from the corresponding cells at earlier stages of gestation in that they were usually flattened and that their nuclei were less often apparent. They contained numerous vesicles and dense bodies not unlike those in the Hofbauer cells seen during the 6th month, but large cytoplasmic vacuoles were only occasionally present.

In material obtained during the 3rd month a second population of cells with distinctive ultrastructural features could be identified, frequently occurring in small groups and possessing numerous processes (Fig. 5). These sometimes partially surrounded masses of cellular debris (Figs. 6, 7) and, in specimens in which blood vessels were present, frequently occurred close to, and occasionally in contact with, cells of the vascular endothelium (Fig. 5). Structures resembling hemidesmosomes were typically present on the plasma membranes of these cells, and at these sites deposits of amorphous material could normally be seen outside the membrane (Fig. 6). The electron density of the cytoplasm was often much greater, and filaments were more abundant and more widely distributed in the cytoplasm (Figs. 7, 8) than in the other cells of the mesenchyme. Irregular areas of further increase in the electron density of the cytoplasm were sometimes seen among the filaments (Fig. 7) and in the region of condensations of filamentous material in relation to the plasma membrane (Fig. 8). Microtubules were also conspicuous, being present in large numbers in the cell processes (Fig. 8) and near the centrioles, which were occasionally visible (Fig. 7).

There was usually marked pinocytotic activity at the plasma membranes (Fig. 7), and vesicles and short segments of agranular endoplasmic reticulum were often present in the cytoplasm. Some cells appeared to contain limited numbers of other organelles, but in others a well-developed Golgi apparatus was present, together with numerous ribosomes and an extensive system of granular endoplasmic reticulum (Fig. 6). Scattered granules, small deposits of glycogen, and lipid droplets were often present in the cytoplasm (Figs. 6, 8).

Elements of this second population of cells were present in the mesenchyme in all material obtained between the 4th and 7th months of pregnancy, and were also conspicuous at term, when they frequently formed the principal cellular component of the mesenchyme. In these older specimens the cells were more evenly distributed in the mesenchyme. Although they invariably had hemidesmosome-like structures on the plasma membranes and numerous lipid droplets and filaments in the cytoplasm, after the 4th month microtubules were no longer visible and there was some reduction in the length and number of the cell processes. Even at term, however, there was still

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Fig. 9. Type 2 cells at term. *hd*, hemidesmosome-like structures on plasma membranes; *mt*, mitochondria; *er*, granular endoplasmic reticulum; *db*, electron-dense body; *cf*, collagen fibrils. Lead and uranyl acetate. × 16000.

Fig. 10. Type 2 cells at term. *hd*, hemidesmosome-like structure; *pcv*, pinocytotic vesicles; *g*, Golgi apparatus; *v*, cytoplasmic vesicles; *va*, vacuole containing dense material. Lead and uranyl acetate. × 25500.

evidence of considerable pinocytotic activity (Fig. 10) and the cells often contained many perinuclear mitochondria (Fig. 9), scattered sacs of granular endoplasmic reticulum (Fig. 9) and a prominent Golgi apparatus (Fig. 10). Numerous vesicles and small vacuoles, some of which contained dense material, occurred in the region of the Golgi apparatus (Fig. 10) and dense membrane-bounded bodies were frequently visible elsewhere in the cytoplasm (Fig. 9). Glycogen was generally abundant, and in some cells filled almost the whole of the cytoplasm.

In the youngest specimens the extracellular tissue consisted largely of scattered collagen fibrils, but by the end of the 3rd month collagen was present in considerably greater quantities and irregular deposits of amorphous material could also be seen in the mesenchyme (Fig. 1). In the later stages of gestation there was a further progressive increase in the amount of collagen and in the oldest specimens and at term the fibrils were generally arranged in the form of a loose network of interlacing bundles (Fig. 5). Isolated masses of cellular debris similar to that seen in the blood vessels and in relation to the second type of extravascular cell during the early stages of their development were also scattered throughout the mesenchyme in material obtained during the 3rd month, and similar material was often present both in the older specimens and at term.

DISCUSSION

The presence of blood vessels in all specimens obtained during the second month is consistent with the early development of a functional circulation throughout the whole of the chorion. The occurrence in this material of structures composed of cells similar to those of the vascular endothelium, but without a definite lumen, suggests that active vasculogenesis sometimes persists in the chorion laeve until the end of the 2nd month. However, blood vessels were absent from much of the material obtained during the 3rd month, and the occurrence, in the remainder, of cellular debris and of cells resembling megakaryocytes in the lumen of the vessels indicates that, even in these specimens, there was already widespread intravascular haemolysis and thrombosis. Although structures resembling at least the remains of blood vessels could be seen in specimens of up to 6 months in age, and although Lister (1968) had described capillary vessels containing blood cells in the mesenchyme of pathological material at term, the present material showed no indication of the persistence of a definitive circulation in the chorion laeve after the end of the 3rd month.

The marked increase in electron density of some of the endothelial cells in specimens obtained during the 3rd month suggests that the changes in the circulation through the chorion laeve are sometimes accompanied by endothelial degeneration. The close similarity between the fine structure of the cells present in the vascular remnants and that of endothelial cells in animals subjected to severe hypoxia (Hasper, 1964) suggests that these also represented stages in the degeneration of cells derived from the vascular endothelium. There was, however, evidence of early degeneration in only a few endothelial cells, and the cells considered to be endothelial in origin formed no more than a small proportion of the components of the vascular remnants. The presence of large amounts of granular endoplasmic reticulum in the periendothelial cells associated with many of the definitive vessels indicates that cells with fibroblast-like functions often contribute to the vessel walls. However, there was no

evidence of the early transformation of any of the components of the vascular endothelium into this type of cell, and the close similarity between the pattern of differentiation of most of the cells of the vascular remnants and that of the extravascular fibroblasts must be regarded as evidence that these structures were mostly formed by cells derived from the periendothelial lining of the larger vessels.

Irregular projections of cytoplasm from the external surface of endothelial cells have been described in vessels present elsewhere in the chorion (Rhodin & Terzakis, 1962). They are particularly numerous, however, in regenerating capillaries (Schoefl, 1963), and since the fine structure of some of the larger projections from the endothelium of the chorionic vessels resembled that of the growing tip of the endothelial sprouts formed in healing tissues (Cliff, 1963), their frequent appearance and prominence in the vessels of the chorion laeve may be related to the early development or retention by the endothelial cells of a capacity for migratory activity. Filaments are also probably a normal feature of the endothelial cytoplasm, but Hills (1964) has shown that they may be more numerous in the cells in ischaemic tissues and Rhodin (1968) has discussed the possibility that their presence in the vascular endothelium may be related to a capacity of the cells for an amoeboid type of motion rather than a role in vascular contraction. Goldman & Follett (1969) have described the development of filaments in association with microtubules and condensations of filamentous material close to the plasma membrane in connective tissue cells during the formation of cell processes *in vitro*; the presence of similar features in the endothelial cells may thus represent additional evidence of the development or persistence in these cells of migratory activity.

The fine structure of many of the isolated cells present in the mesenchyme was similar to that of the mesenchymal cells of the amnion (Hoyes, 1970), and the close relation of the differentiation of these cells to the appearance of extracellular deposits of amorphous material and to a marked increase in the amount of collagen indicates that they were also largely fibroblastic in nature. In specimens obtained during the 3rd month the components of a second distinct population of cells could be identified in the mesenchyme. Although these were in some respects similar to the cells considered by Parry (1970) to produce elastin, there was evidence that at least a proportion were vascular in origin. The appearance of these cells in the mesenchyme was closely related to the disappearance of the circulation, and, during the 3rd month, they were found close to masses of cellular debris not unlike that seen in the vascular lumen. In material in which blood vessels were still present, these cells were seen both close to and in contact with the vascular endothelium, and the presence in their cytoplasm of large numbers of microtubules and considerable amounts of filamentous material suggests that they were capable of the same kind of migratory activity as that considered to exist in the vascular endothelium. The hemidesmosome-like structures characteristically present on their plasma membranes were not apparent in the fibroblasts, but were also seen in the endothelial components of the vascular remnants, and it appears not unlikely that these represented the persistent remains of the areas of membrane specialization which previously formed the attachment plaques between adjacent endothelial cells.

The ultrastructural features of the cells of this second type can also be related to those of the pericytes and some of the other cells present in the walls of blood vessels.

Areas of increased electron density resembling those present among the filaments of smooth muscle cells could sometimes be demonstrated in the filaments contained in the cytoplasm of the cells, but there was no evidence of their transformation into fully developed muscle cells, and similar structures were also seen among the filaments present in the fibroblasts. The frequent occurrence of considerable amounts of endoplasmic reticulum indicates that the cells also retained a potential for some of the other types of differentiation postulated for the vascular pericyte (Movat & Fernando, 1964; Rhodin, 1968), and the presence of marked pinocytotic activity at their plasma membranes, both during the early stages of their development and at term, is suggestive of a capacity to absorb materials from the extracellular space. Rhodin (1968) has recently suggested that the existence of such activity in the mural pericyte is related to the detection and sensitization of the vascular endothelium to substances such as histamine and serotonin released into the extravascular space. It is possible that, by removing these and other substances derived either from the cellular debris present in the mesenchyme or from the maternal tissues, this second type of mesenchymal cell prevents their transmission to the amniotic cavity and ultimately to the fetus. The lipid droplets in these cells may also have been formed from lipid derived from degenerate mesenchymal elements or, since the droplets were prominent in areas in which they were also numerous in the overlying epithelium and decidua, from the maternal tissues. The progressive increase in the amount of glycogen in their cytoplasm is consistent with a role of these cells in the storage of carbohydrate, and the occurrence of dense bodies in their cytoplasm in the older specimens may be regarded as evidence of the absorption and sequestration of other materials, such as protein. The presence of dense material in vacuoles related to the Golgi apparatus is suggestive of the development of secretory activity in some of the cells of this type, and it is possibly significant that the mesenchymal cells of the chorion laeve have recently been implicated in the production of renin (Symonds *et al.* 1970).

A large Golgi apparatus and considerable numbers of dense bodies have also been described in mesenchymal cells of the placental chorion (Enders & King, 1970); these cells frequently occur within a sheath of connective tissue cells not unlike those forming the periendothelial lining of the blood vessels and much of the wall of the vascular remnants present in the chorion laeve, and this suggests that they represent further components of the specific population of absorptive cells seen in the regions of the chorion. Cells capable of such functions may therefore be present throughout the chorion, and it is possible that the cells which possess many of the histochemical features of macrophages (Fox & Kharkongor, 1969) and the ability to take up neutral red (Lewis, 1924) and meconium (Bourne, 1962) are also elements in this population. Such cells are generally classified as Hofbauer cells, and the occurrence of the cytoplasmic vacuolation, which is their principal diagnostic feature, in the cells described by Enders & King (1970) appears to provide additional support for such a classification. However, there is now considerable evidence that the development of such vacuoles may be related to the occurrence of processes other than those involved in the uptake of the kind of materials considered to be absorbed by this type of cell (Fedorko, Hirsch & Cohn, 1968*a*, *b*; Tapp, 1969, 1970; Glauert, Fell & Dingle, 1969) and the present investigation has provided further support for the findings of a previous study on the amniotic mesenc hy π ϵ (Hoyes, 1970), in which it was suggeste

that the Hofbauer cells principally consist of degenerate mesenchymal elements, and are largely formed from the stromal fibroblasts.

SUMMARY

The fine structure of the mesenchyme associated with the chorion laeve was studied in specimens obtained between the 2nd and 7th months of pregnancy and at term. Capillary vessels containing fetal blood cells were present in specimens of up to 3 months in age. In those seen in material obtained during the 3rd month there was evidence of intravascular haemolysis and thrombosis, and a definitive circulation could not be demonstrated in the chorion laeve after the end of this month. Vessel-like structures were apparent in the mesenchyme in specimens of up to 6 months, but consisted mostly of cells with ultrastructural features similar to those of the extravascular fibroblasts. They were thought to be composed principally of derivatives of the fibroblast-like periendothelial cells lining the larger vessels. Other cells occasionally present in these structures were regarded as degenerate endothelial elements and there was some other evidence of early endothelial degeneration. There was also evidence of the early development, in many of the endothelial cells, of a capacity for migratory activity, and it was suggested that the components of the morphologically distinct population of cells which could be demonstrated in material obtained during the 3rd month were largely vascular in origin. The fine structure of these cells was also related to that of some of the other types of cell present in the walls of blood vessels and their functional activity and relationship to the cells variously classified as Hofbauer cells was discussed.

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