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Fine Structure of Multiple Neonatal Haemangioendothelioma of the Liver

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Summary. This paper discusses the fine structure of multiple haemangioendothelioma of the liver. There have been no previous reports of electron microscopic studies of this tumour, which was found to be composed of young capillaries continuous in type and varying in calibre. The capillaries were lined by actively proliferating, immature endothelial cells with pericytes forming an integral part of the tumour. Their morphological characteristics indicated intensive protein production. The authors suggest that the active pericytes play a role in the production of reticular fibres of the tumour.

Following corticosteroid therapy, regression of tumour nodules occurred in this case in the interval between surgery and autopsy. This was confirmed by histological studies. The results seem to support the hypothesis that actively proliferating, young endothelial cells show an increased sensitivity to corticosteroids.

Key words: Endothelial cells – Pericytes – Basement membrane – Reticular fibres.

Multiple neonatal haemangioendothelioma of the liver is a rare developmental anomaly. About 60 cases have been reported (Rocchini et al., 1976), and in general they show vasoformative neoplastic foci in the liver which, although they have no capsule – are separated from the liver tissue (Willis, 1967). Within the tumour arteriovenous shunts may develop resulting in congestive heart failure (Touloukian, 1970; Dehner and Ishak, 1971; McLean et al., 1972). Histologically, the tumour is benign but in spite of this prognosis is poor. Several methods of treatment have been attempted, i.e. irradiation, surgical excision, ligation of the hepatic artery (Blumenfeld et al., 1968; Laird et al., 1976), all with unsatisfactory results. Recently the dramatic effect of corticosteroid therapy has been pointed out (Touloukian, 1970; Rocchini et al., 1976).

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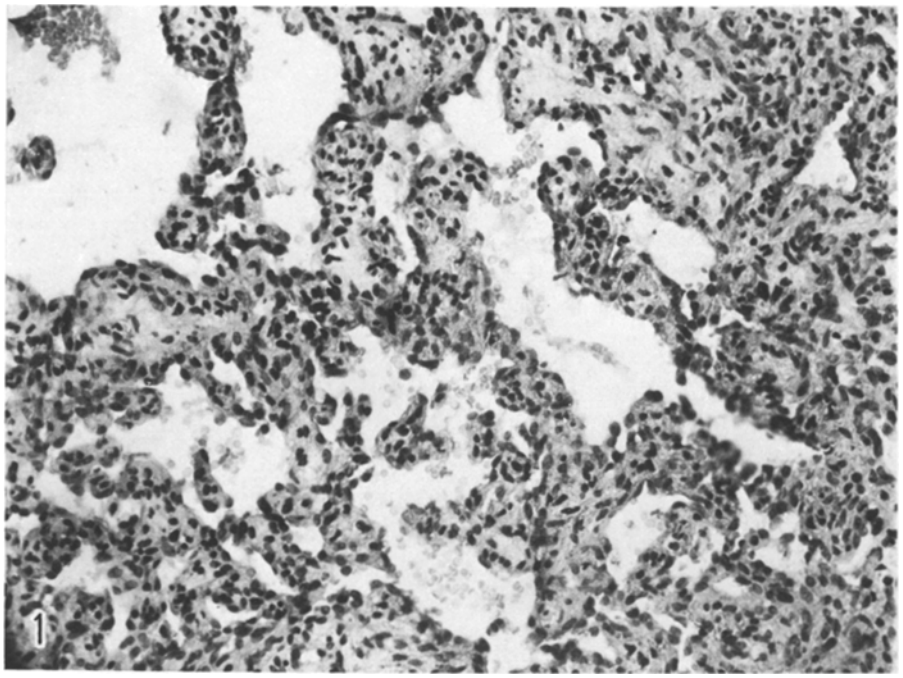


Fig. 1. Light microscopy. The tumour consists of blood vessels varying in calibre. The tumour is abundant in cells, the endothelial cells are large (×140)

The light-microscopic features of the tumour have long been known (Willis, 1967; Dehner and Ishak, 1971; McLean et al., 1972; Braun et al., 1975). Results of electron-microscopic studies have, however, not been published.

Case Report

G.G., infant girl. Birthweight 2,700 g. Two weeks after birth she was hospitalized for pneumonia and found to have an enlarged, firm liver extending two fingerbreadths below the costal margin. On 20 Febr. 1976, at the age of $2^1/_2$ months she was admitted to our hospital for anaemia, pneumonia and increased hepatomegaly. At that time, a large, bulging abdomen with dilated veins was found. Examination of the blood showed: RBC: 1,900,000 Hgb: 6.3% WBC: 10,400. Differential count: SE 22, Eo 2, Ly 71, No 4, Pl 1. Thrombocytes: 210,000. Liver function tests were normal on. Liver scintigraphy there were numerous, nut-sized, circumscribed, inactive areas in both lobes.

With improvement of the pneumonia, laparotomy was performed. In both lobes of the liver, multiple, protruding, round, soft, greyish-white tumours, measuring 3–6 cm in diameter were found. From one of these a wedge biopsy was obtained.

Histological Findings: The tumour was composed of loosely arranged blood vessels in scanty connective tissue stroma (Fig. 1). The lumen of the vessels varied in diameter: cavernous, dilated blood-

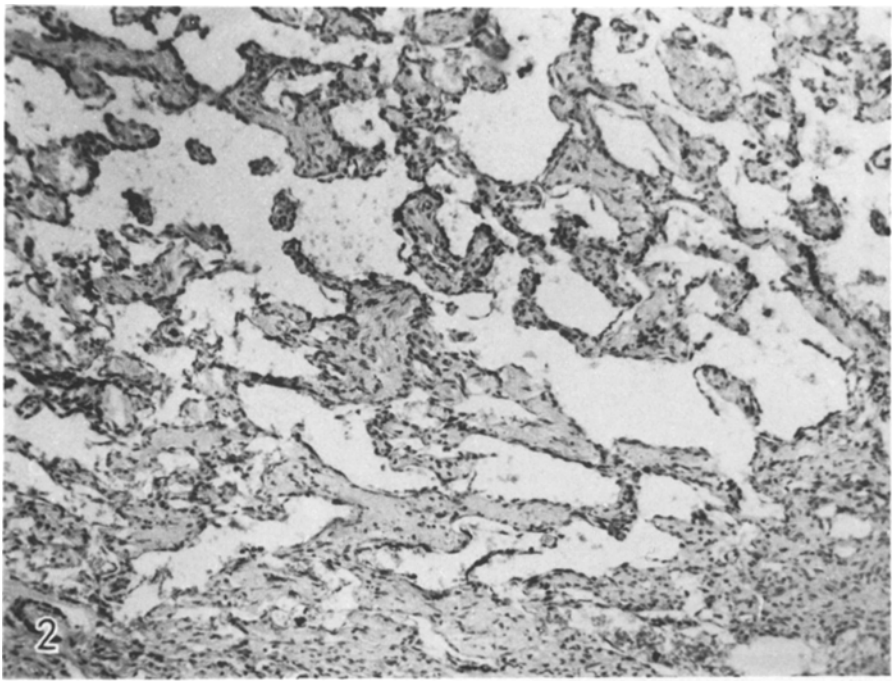


Fig. 2. In the autopsy material the cellularity has diminished, the endothelial cells are flat, the stroma is scarred (× 120/)

spaces were found as well as capillary buds with little evidence of a lumen. The vessels were lined by swollen endothelial cells. In the stroma bile ducts could be identified. A diagnosis of Neonatal hepatic haemangioendothelioma was made .

Sixteen days after surgery the patient developed mesenteric thrombosis with small intestinal necrosis and underwent small bowel resection and anastomosis. Five days later a stercoral fistula developed. As a consequence corticosteroid therapy (8 mg/kg) die Prednisolon—prescribed for the liver tumour—was discontinued after 50 days, and she was subjected to X-ray irradiation (1200 R). Following corticosteroid and irradiation therapy the inreasing hepatomegaly was arrested but the stercoral fistula persisted. Despite intensive parenteral feeding, her body weight decreased rapidly and she died at the age of 6 months.

At autopsy (Dr. G. Gorácz) the cadaver weighed 3,400 g. Numerous tumour nodules, $^1/_2$–2 cm in diameter, protruding slighty from the liver surface were found. Some of these were purplished, soft and spongy, others were greyishwhite and firm. Intravenous cannulation resulted in thrombophlebitis of the bracho-cephalic, vein and superior vena cava, giving rise to septic pulmonary infarcts and purulent meningitis.

Microscopic examination of the autopsy material showed a definite decrease of cellularity of the liver tumour. Large areas of scar tissue were formed. The capillary buds, having fairly narrow lumina, had almost completely disappeared. The endothelial cells of the cavernous blood vessels were flat (Fig. 2).

Method of Electron Microscopic Study: The material was fixed in 2% osmium tetroxide, buffered according to Palade, dehydrated in alcohol, embedded in Araldite. The sections were prepared by Reichert Ultramicrotome and examined by JEM 100 B electron microscope. For orientation semi-thin (0.5 n) sections stained with toluidine blue were prepared.

Results

In agreement with the light-microscopic picture, the calibre of the vascular elements of the tumour varied. Vessel-buds with small lumina could be found (Fig. 3), cavernous slit-like blood spaces (Fig. 4) and areas in which the endothelial cells of the tumour were scattered between the cells of the stroma were all found (Fig. 5).

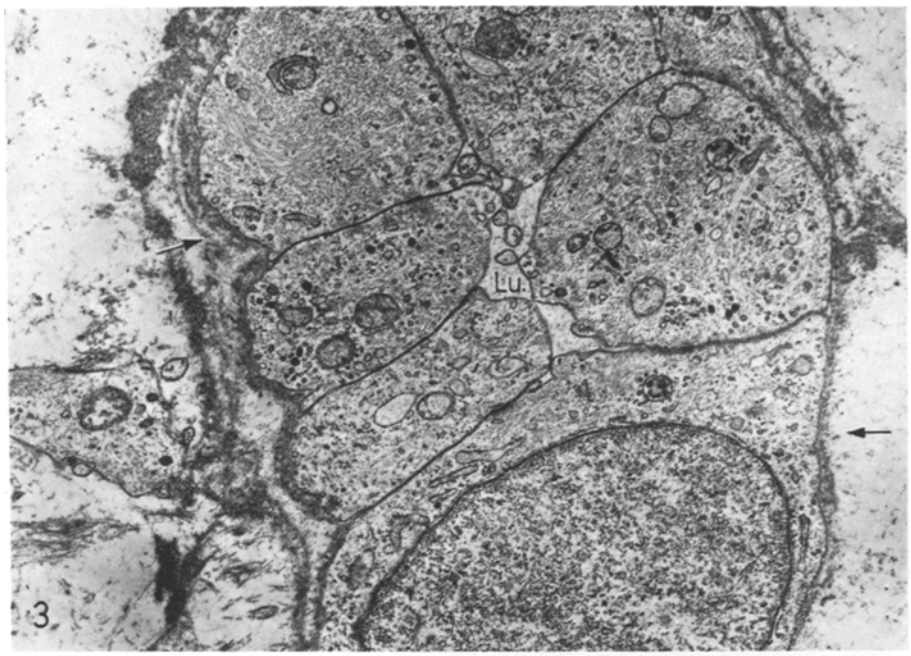
Endothelial Cells

The main mass of the tumour was formed by cuboidal endothelial cells protruding into the lumina of the vessels and forming a continuous lining. The cell-nuclei were large and oval shaped. Occasionally, large, bizarre shaped, deeply indented nuclei could also be found. The cells were irregular in shape and the marked plasticity of the cell membrane was striking. Numerous cytoplasmic protrusions were seen on the lumenal surface of the cells but occasionally also on the outer aspect of the cell membrane (Fig. 6). Numerous filaments, varying from 50 to 70 Å in thickness, could be found in the cytoplasm of the endothelial cells, partly in a reticular arrangement, partly in bundles (see Figs. 3. and 9.). In some of the cells there were hardly any cell organelles apart from the filaments (Fig. 9). In others, numerous mitochondria, rough endoplasmic reticulum and an extensive Golgi zone were be observed. The mitochondria varied in size and shape and their cristae were disorganized (Figs. 7, 8). In the vicinity of the Golgi zones numerous vesiculae could be observed (Fig. 8). There was a surprisingly small number of pinocytotic vesicules – most of them situated on the opposite side of the cell to the lumen, along the basement membrane (Fig. 7).

The cell junctions showed different stages of development. In some areas they formed a continuous link among the endothelial cells. In such regions the intercellular space was narrow (Fig. 3). In others, the structures were absent in long segments with the endothelial cells loosly arranged (Figs. 4. and 8). In the endothelial cells, in some places, characteristic Weibel-Palade (tubular-lamellar) bodies could be observed (Fig. 6).

Pericytes

In the tumour tissue numerous pericytes could be observed which could be easily differentiated from the endothelial cells. Generally they were located in the immediate vicinity of the endothelial cells with only the basement membrane between. The cell nucleus was oval shaped. Occasionally several glycogen particles were found in their cytoplasm (Fig. 10). They contained few mitochondria, moderately-developed Golgi zones, a large amount of rough endoplasmic reticula and free ribosomes (Fig. 11). The rough endoplasmic reticulum showed several cystern-like dilatations. These saccules contained fine granular and, occasionally homogenous, electron dense material (Fig. 12).



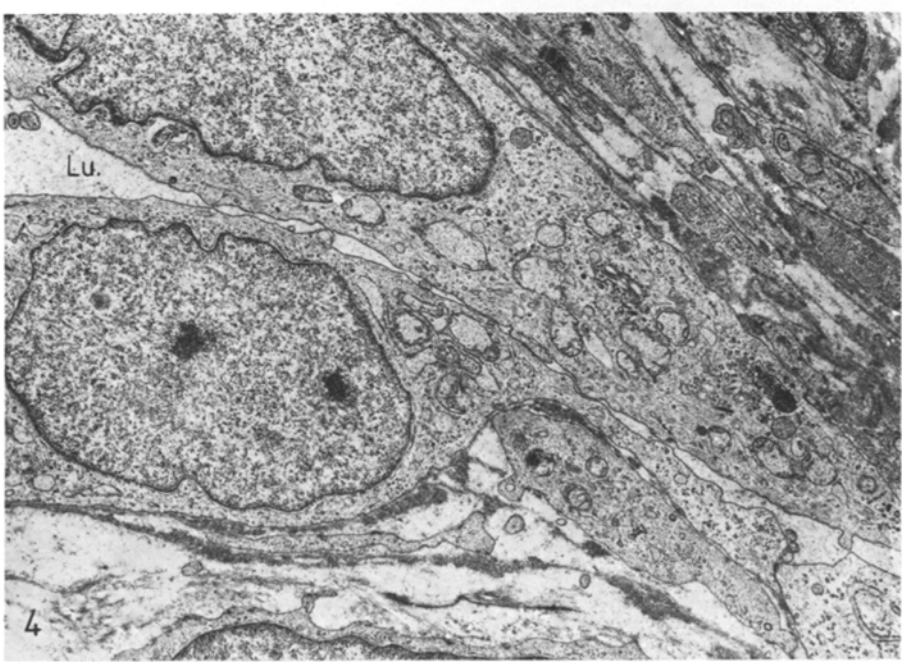
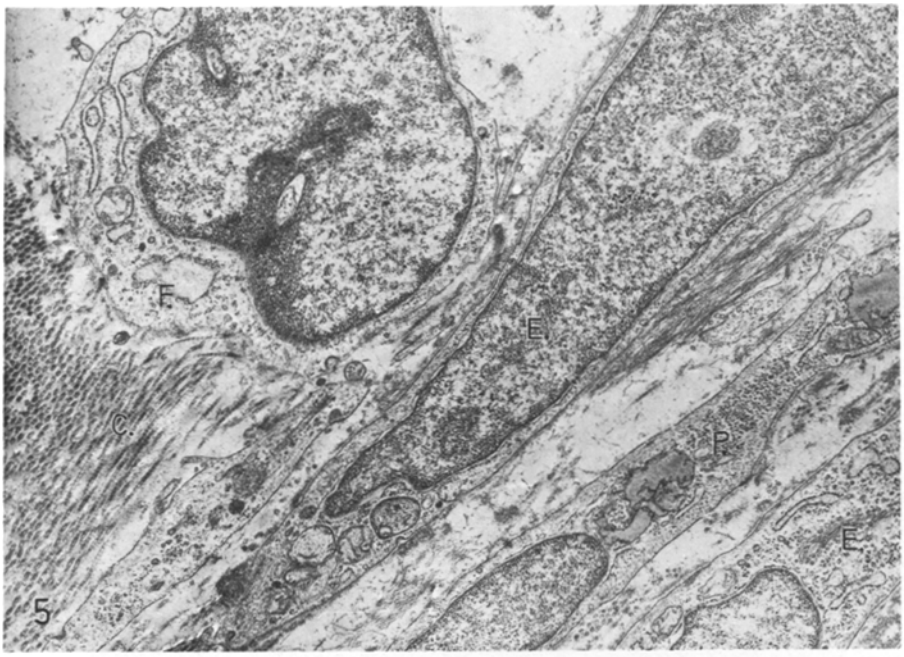


Fig. 3. A capillary bordered by six swollen endothelial cells. The lumen (*lu*) is narrow, the basement membrane varies in thickness (arrows) (× 12,500)

Fig. 4. Section of a larger-calibre capillary with slitlike lumen (*lu*) and elongated endothelial cells (× 8250)



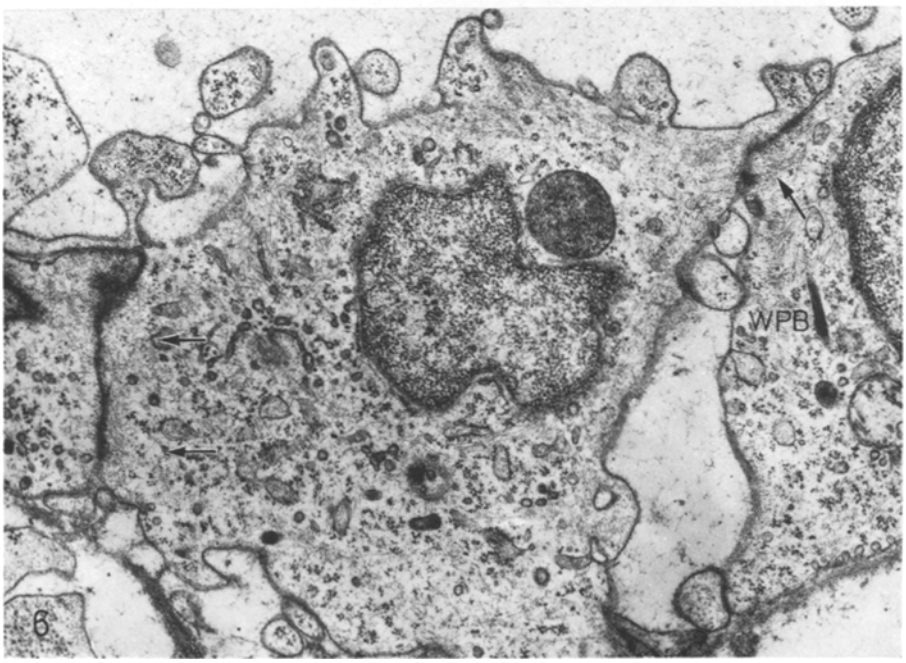


Fig. 5. Section of two endothelial cells (*E*) in the vicinity of fibroblast (*F*) and pericyte (*P*). The cells are in loose contact, there is no evidence of a lumen (× 14,650)

Fig. 6. Neoplastic endothelial cells. Protrusions arising from the surface of the endothelial cell, bulging into the lumen of the vessel. On one side of the cell, it is joined to the neighbouring cell by a well-developed cell-binding structure. On the other side, these junctions are only visible on a short segment (arrows). On the right, the tubular body of Weibel and Palade (*WPB*) can bee seen (× 16,750)

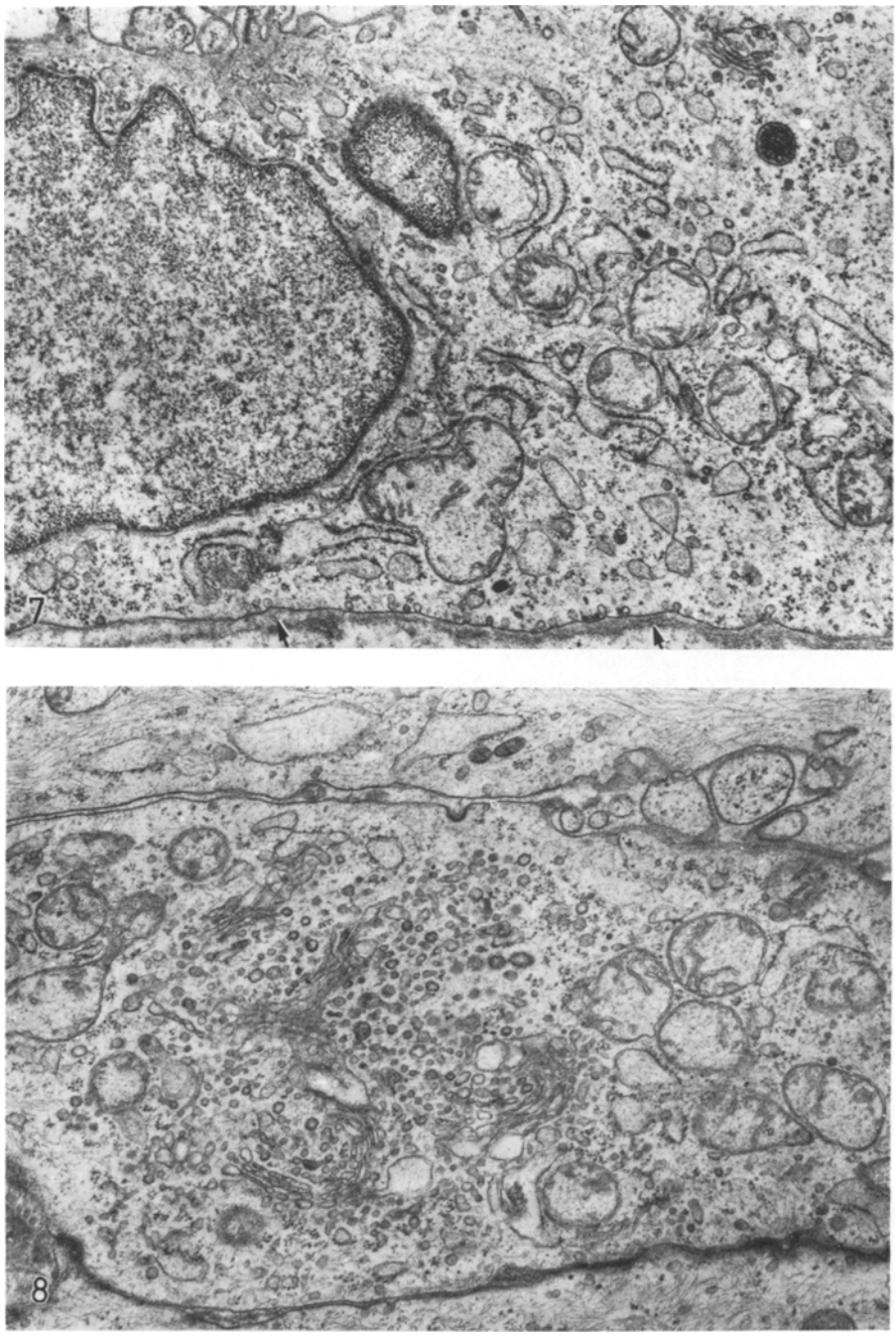
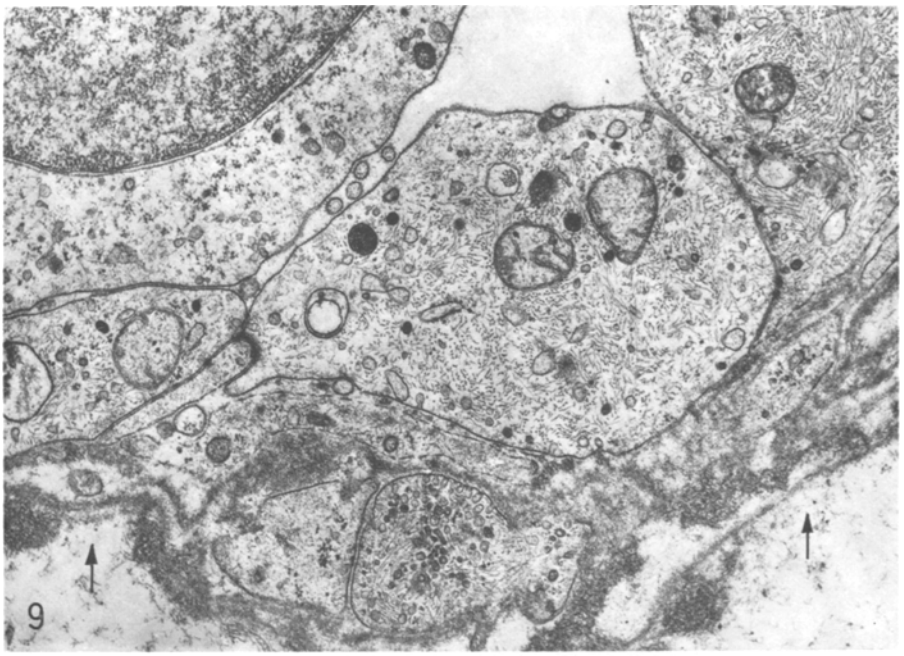


Fig. 7. In the cytoplasm of the endothelial cells there are swollen mitochondria, rough endoplasmic reticulum and free ribosomes. On the opposite side of the lumen, along the basement membrane, pinocytotic vesicules can be observed (arrows) (× 16,750)

Fig. 8. In the neoplastic endothelial cell, extensive Golgi zone with numerous vesicules can be observed (× 30,000/)



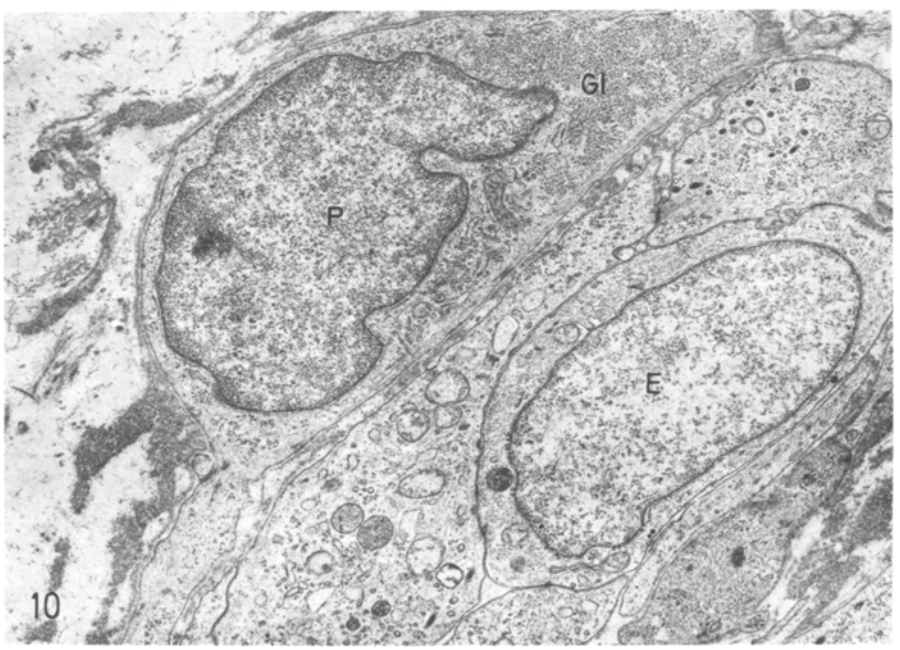


Fig. 9. Loosely connected endothelial cells without cell binding structures. The basement membrane is irregular and of several layers in depth (/arrows/) (× 16,750)

Fig. 10. Pericyte (*P*) easily distinguished from the endothelial cells (*E*). The cytoplasm contains glycogenous granules (*Gl*). (× 14,650)

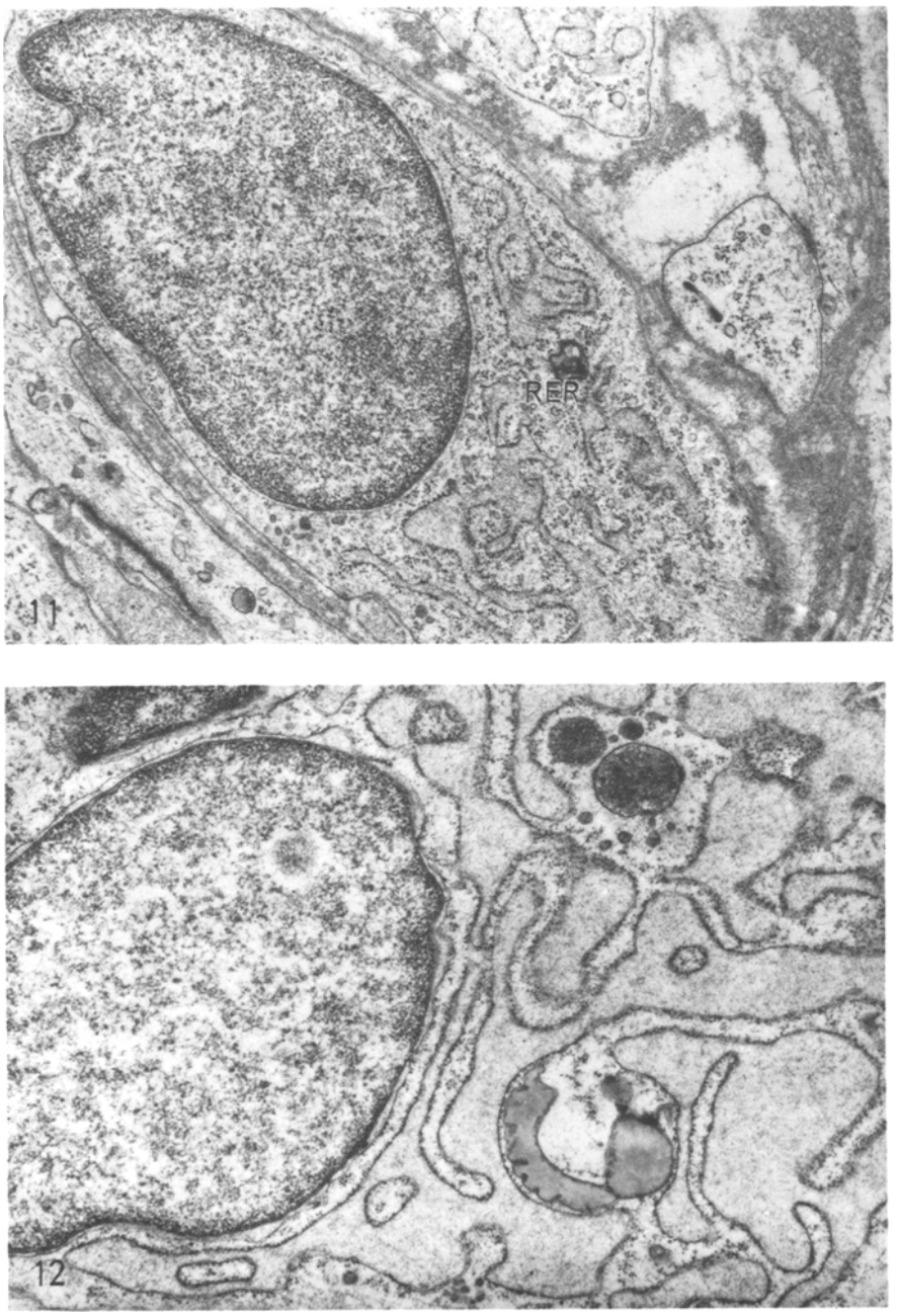


Fig. 11. Pericyte surrounded by basement membrane with numerous parallel rough endoplasmic reticula (*RER*) and free ribosomes in the cytoplasm. In the area indicated by arrows there is a deposit composed of a substance similar to the basement membrane (× 16,750)

Fig. 12. In the cytoplasm of a pericyte the lamellae of the rough endoplasmic reticulum show cysternlike dilatation and contain fine granular material (× 30,000)

Basement Membrane

The blood vessels of the tumour were surrounded by a basement membrane of varying thickness (300–500μ), and, in many places several layers in depth (Fig. 9). This basement membrane could be observed around all the vessels, both the small and the cavernous type, being absent only where the endothelial cells occured freely, embedded in the stroma. All the pericytes were surrounded by basement membrane. Granular-fibrillar bundles with similar density to that of the basement membrane could be observed everywhere in the tumour (Figs. 4, 9, 10). Semi-thin sections suggested that these represent the reticular fibres of the tumour.

Discussion

Multiple neonatal haenangioendothelioma of the liver differs both morphologically and clinically from the haemangioendothelioma of the liver in adults. The neonatal hepatic haemangioendothelioma has three components (McLean et al., 1972):

1. Blood vessels 100–400 μ in diameter with flat endothelial cells.
2. Vessels 20 μ in diameter with a high endothelial cell lining.
3. Solid groups of mesoblastic, primordial cells.

The three forms are considered to represent various degrees of proliferation and involution. Despite the high degree of cellularity, the histological picture in our case was benign. Cellular atypia, abnormal mitotic figures and giant cells could not be seen, in contrast to the usual findings in the liver haemangioendothelioma of adults (Danz et al., 1970; Ludwig and Hoffmann, 1975).

In our case, the light and electron-microscopic studies showed that the endothelial cells lining the tumour vessels formed a continous layer, i.e. they were continuous capillaries (Simon, 1966; Bruns and Palade, 1968).The endothelial cells were larger than normal, their nuclei were larger, and the cytoplasm more abundant. Their shape was irregular with numerous processes towards the lumen and the lateral surface. Similar large endothelial cells with extensions and rich in organelles have been described in regenerating vessels (Schoefl, 1963), in skin haemangiomas of infants (Fuchs, 1967) and in nasopharyngeal angiofibromas (Stiller et al., 1976).

Between the endothelial cells poorly-developed cell junctions were seen. Occasionally, these were absent and in these areas loose connection of the tumour cells was evident. The early stage of development of the cell junctions was also observed in regenerating capillaries (Schoefl, 1963), in the vessels of oral giant-cell granuloma (Andersen et al., 1975) and in the skin lesion of lupus erythematosus (Haustein and Klug, 1975).

The cylindrical, electron-dense tubular-lamellar bodies surrounded by a single membrane – discovered by Weibel and Palade (1964) – are characteristic formations in endothelial cells as demonstrated also in our material. Their importance is still not known although their morphological appearance resembles lysosomes. They have been described in different animal species as well as in humans. They have also been shown to occur in human tumours, among them senile

cutaneous angiomas. In our case, slight pinocytotic activity of the endothelial cells was evident.

In the cytoplasm of the endothelial cells numerous fine fibrils could be seen, some of them in reticular arrangement, others in bundles. The cytoplasm of some of the cells was almost completely filled with fibrils. Large numbers of fibrils have also been described in haemangioendothelial sarcoma of bone (Steiner and Dorman, 1972) and in capillary haemangioma of the skin in infants (Fuchs, 1967).

In other regions, several swollen mitochondria, parallelly arranged rough endoplasmic reticula and extensive Golgi zones, with numerous vesicles in the vicinity of the latter were seen in tumor cells.

Our investigations showed that the pericytes also formed an integral part of the tumour. They were larger than the normal pericytes as in cases of haemangiopericytoma (Ramsey, 1976; Murad et al., 1968; Silverberg et al., 1971). In the cytoplasm of these pericytes extensive rough endoplasmic reticulum in parallel arrays was evident, containing a finely granular substance. This morphological picture suggests intensive protein-producing activity, an appearance also observed by Brommer et al. (1976). In cases of haemangiopericytoma these authors assumed that pericytes play an important role in producing the substance of the basement membrane and of reticular fibres. In our case, the pericytes and tumour vessels were surrounded by a wide irregular basement membrane, frequently composed of multiple layers. Multiplication of the basement membrane has also been observed in senile angioma of the skin (Stehben and Ludatscher, 1968) and in haemangio-endothelio-sarcoma of the bone (Steiner and Dorman, 1972). The finely granulated, moderately electron dense deposits, similar to the basement membrane material was seen everywhere in the tumour. With the exception of pericytes, no cells were present with an appearance suggesting intensive protein production. Thus Steiner and Dorman's (1972) hypothesis in which the active pericytes play an important role in the formation of reticular fibres of the tumour, seems reasonable.

In our case regression of the tumour nodules was observed in the interval between surgery and autopsy following steroid therapy. This regression was confirmed by light-microscipic examination. A beneficial effect of corticosteroids is supported by clinical observations (Zarem and Edgerton, 1961; Touloukian, 1970; Schiliro et al., 1976). Theoretically, it might be based on the experimental finding that the sensitivity of vessels to circulating vasoconstrictive substances is increased by corticosteroids (Zweifach, 1953). According to Zarem and Edgerton (1961), immature vascular tissue is particularly sensitive to the antianabolic effect of corticosteroids. This observation is supported by our study.

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