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Morphologic Considerations on the Placenta in Congenital Nephrotic Syndrome of Finnish Type

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Summary. We have studied the morphological aspects of a thirty-six week gestational age placenta in the Congenital Nephrotic Syndrome of Finnish type. The study, conducted with histological, histochemical, morphometric and ultrastructural methods, demonstrates the presence of primary disorders of placentation consisting of persistent embryonic villi, arrested ramification and chorionangiomatosis. The villous development is compatible with the first-second trimester of pregnancy. Vasculo-syncytial membranes are quantitatively increased. Histochemical findings document placental immaturity further: Perls' reaction was positive for the trophoblast basement membrane (this is normally not observed beyond the second trimester), Alcian Blue positivity at pH 1 was also evident and was observed in three month gestational age placentae and in controls. Periodic Acid Silver Methenamine and Thioaldehyde Fuchsin documented abnormal thickenings of the trophoblast basement membrane. Electron microscopic observation reveals that the trophoblast basement membrane is thickened. Osmiophilic bodies are distributed throughout the trophoblast basement membrane and also within the basement membrane like material. Abundant microfibrils are present in the villous stroma. Lamination of basement membrane like material is observed in a subendothelial position. On the basis of their findings and in conjunction with the data in the literature regarding biochemical alterations of renal glomerular basement membranes in Congenital Nephrotic Syndrome Finnish type, the Authors suggest that a primitive membranopathy forms the basis for this pathological condition.

Key words: Congenital Nephrotic Syndrome of Finnish type – Histochemical – Placentomegaly – Trophoblast basement membrane alteration – Ultrastructure.

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Placentomegaly is a characteristic finding in the Congenital Nephrotic Syndrome of Finnish type (CNF)[1]. To date, the macroscopic and light microscopic observations in this field have centered upon one case involving a full-term placenta (Kouvalainen et al. 1962) and other studies involving several placentae obtained from therapeutically aborted first-trimester pregnancies (Wiggelinhuizen et al. 1976). We have conducted this study on a thirty-six week old placenta from a case of CNF, using morphological, morphometric, histochemical and ultrastructural analyses.

Case History

The obstetric history of a 25 year old Italian woman[2] included two previous pregnancies which have been extensively reported in earlier studies (Gemelli et al. 1977). The clinical course of the present (third) pregnancy was normal. Human placental lactogen (HPL), oestriol (E_3), and alpha$_1$-fetoprotein (aFP) were evaluated at weekly intervals from the twelfth week to term by serum radioimmunological assay and found to be within normal limits. Amniocentesis procedures for the determination of αFP were unavailable at the time. The delivery was at weeks with a podalic presentation.

The placenta, weighing 1.4 kg, measuring $20 \times 15 \times 5$ cm, was friable. The maternal aspect presented large blue-red cotyledons. The umbilical cord, centrally attached, was somewhat oedematous and thickened. Serial sections, at two centimeter intervals, demonstrated only diffuse tissue oedema. The umbilical vasculature was normal.

The present newborn weighed 2.2 kg at birth and the placentofetal ratio was 0.65. From the first day of life the neonate presented with non-selective proteinuria, microhaematuria, hypoproteinaemia and hyperlipidaemia. On the ninteenth day moderate oedema was noted and on the forty-fifth day assumed a diffuse character. The neonate was transferred on the fifty-third day to a Pediatric Nephrology center where the clinical diagnosis of CNS was characterized as Finnish type after biopsy. The neonate is currently alive despite developmental and psychomotor deficits.

Material and Methods

The placenta was placed in buffered formalin pH 7.2 according to Karnowsky (1965). After several days, the placenta was sectioned into vertical strips each of which was divided into central and peripheral zones. Tissue sections were taken from each zone subsequent to its subdivision into subchorionic, intermediate, and maternal areas as per Fox' procedure (1964). Routine paraffin sections were stained with haematoxylin and eosin and Azan-Mallory.

Quantitative study of vasculo-syncytial membranes (VSM) and syncytial knots (SK) were also performed according to Fox (1965, 1967). On four sections stained with haematoxylin and eosin, one hundred terminal villi of the maternal subdivision were counted in each section. The number of VSM and SK was expressed as a percentage of the total.

The following *histochemical procedures* were carried out: Alcian Blue (AB) pH 1 (Lev and Spicer 1964) and pH 2.5 (Mowry 1956), Perls' Prussian blue reaction, PAS, Periodic Acid Silver Methenamine (PASM), Thioaldehyde Fuchsin (TAF) (Bussolati and Bassa 1974), Congo Red and Van Kossa. Placentae obtained from induced abortions at three months and one from a normal pregnancy (36 weeks) were used as controls.

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[1] CNF, or "microcystic disease", is a rare form of the group of congenital nephrotic syndromes (CNS) characterized by onset within the first months of life (Hallman et al. 1967; Hoyer et al. 1967; Grislain et al. 1973; Habib and Bois 1973; Royer et al. 1975; Huttunen 1976). The original designation "Finnish type" reflects the high incidence of this disorder in Finland encountered in 85 pro 137 cases of CNS (Bulla et al. 1974) and with a frequency of 1:8,200 births (Huttunen 1976). Recently, we reported the first Italian case with CNF (Gemelli et al. 1977)

[2] Both this woman and her husband are natives of Calabria, not consanguinous, and apparently healthy

On *frozen sections*, direct immunofluorescence was performed using anti-IgG and anti-C_3 sera (Behring Institute) labelled with fluorescein isothiocyanate (FITC). Appropriate controls were also employed. Observations were carried out using a Zeiss microscope equipped with an Osram HB 200 mercury lamp, excitation filters BG 12 and BG 38 and arrest filters no. 44 and no. 53.

Small tissue fragments, initially fixed in formalin, were reprocessed through glutaraldehyde and 1% OsO_4 using 0.1 M phosphate buffer at pH 7.4, dehydrated in alcohol and embedded in Araldite. Epoxy semi-thin sections were prepared by Giemsa, PAS, and PASM. Ultrathin sections were contrasted with uranyl-acetate and lead citrate and observed under a Siemens transmission *electron microscope* (TEM) Elmiskop II at 80 kw tension.

Results

Light Microscopic Findings

The overall pattern described below was observed in subchorionic, intermediate and maternal areas of the placenta. Few interspersed terminal villi are normal and fully developed; most of the terminal villi appear enlarged with oedematous embryonic connective tissue (Fig. 1 a). Their syncytialtrophoblast covering layer is irregularly thinned. Cytotrophoblast cells are still present in many villi. Intervillous bridges are rare. The intervillous spaces are wide and contain occasional microdeposits of perivillous fibrinous material. There are numerous Hofbauer cells present in the villous stroma. Other terminal villi are plump, contain histiocytes and Hofbauer cells in a wide meshwork of villous stroma. In this instance, villous bridges are present. Villous vascularity is extremely variable. Some villi have an insufficient number of narrow peripherally oriented capillaries. Other villi show, in a reticular stroma, proliferating closely arranged capillaries, predominantly of the sinusoidal type; among these latter, voluminous microaneurysma capillaries are observed, having a diameter of greater than 100 microns (Fig. 1 b).

The maternal zone VSMs are clearly evident in well vascularized villi (Fig. 1 c). The morphometric data regarding VSMs and SKs are reported in Table 1.

Primary and secondary villi, more frequently encountered in subchorionic and intermediate zones present with: (1) a variable degree of reticular stromal oedema, (2) centrally positioned cavernous vessels and (3) a stratum of connective tissue fibers closely apposed to the vessel wall.

Inflammatory processes or infarcts were not observed.

Semi-thin sections of terminal villous trophoblast show a loose connective tissue matrix forming hydrophilic lacunae; the capillary endothelium is particu-

Table 1. Incidence of vasculo-syncytial membranes (*VSM*) and syncytial knots (*SK*) in a placenta delivered at 38 weeks gestation in a case of CNS

Percentage of villi with VSM		Percentage of villi with SK	
Case report	Normal[a]	Case report	Normal[b]
52	(6–30)	15	(11–30)

[a] Fox (1967)
[b] Fox (1965)

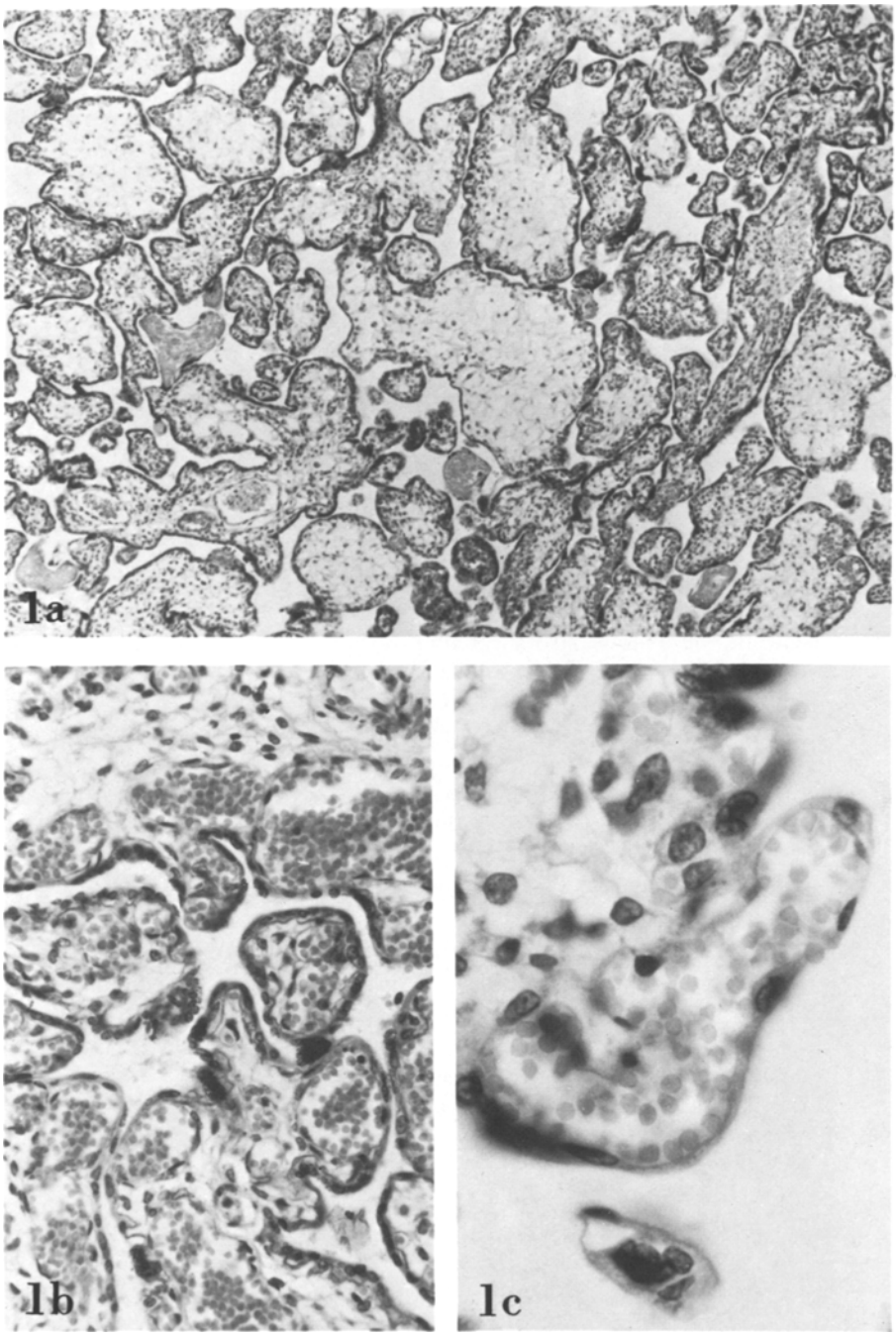

Fig. 1. a Oedematous embrionic villi and interspersed plump villi. Note the high cellularity of plump villous stroma (H & E, × 100). **b** Exhuberant vessel proliferation with sinusoidal or microaneurysmal capillaries in embryonic villous stroma (H & E, × 360). **c** Extensive vasculo-syncytial membrane in a prominent sinusoidal capillary (H & E, × 600)

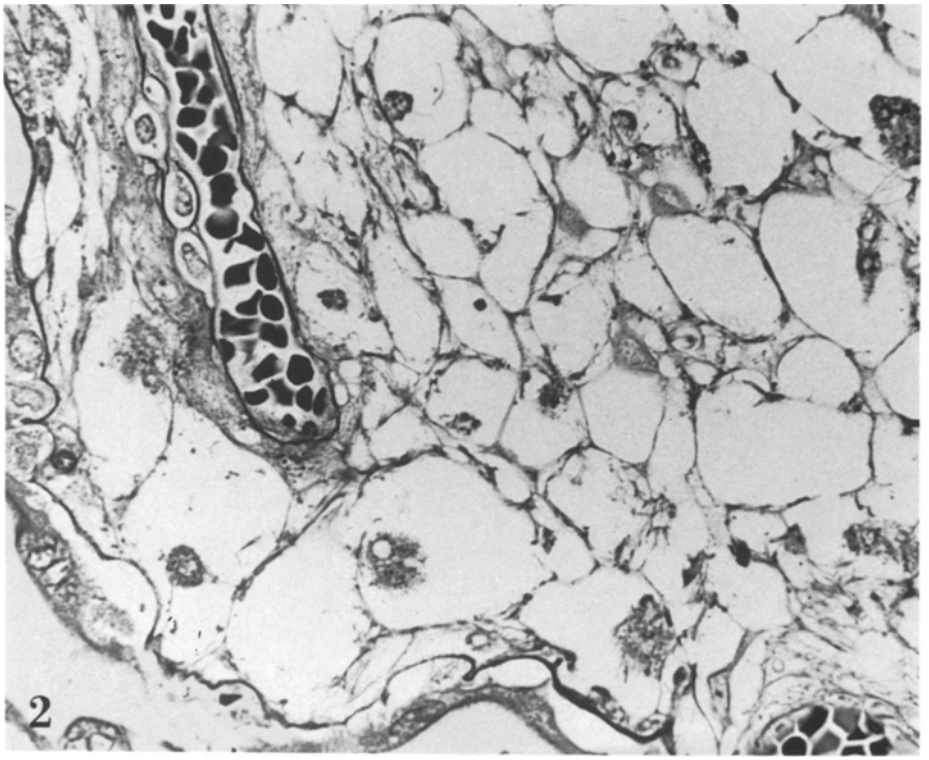

Fig. 2. Stromal oedema in a wide reticular meshwork with Hofbauer cells. Note endothelial cell hypertrophy (Giemsa, ×900)

larly prominent and surrounded by a clearly identifyable basal membrane (BM) (Fig. 2).

Histochemical Findings

Staining with AB pH 1 demonstrated a positive reaction of the trophoblast BM with areas of thin and widened segmental reactivity. AB pH 2.5 was negative for the trophoblast BM and tenuous for the stroma. In the three month old control placentae AB pH 1 demonstrated, positivity of the trophoblast BM and the stroma, whereas AB pH 2.5 resulted in a very tenuous reaction at the same levels. In the thirty-six week apparently normal control placenta AB pH 1 and AB pH 2.5 were both extremely weak.

Employing Perls' method one observes an intense positive reaction of the villous trophoblast BM. This positivity is fundamentally uniform but shows occasional granular localization and includes slightly wider areas of stain uptake (Fig. 3a). A weak positive Perls' reaction is occasionally encountered in the villous stroma. In first trimester control placentae Perls' reaction demonstrates

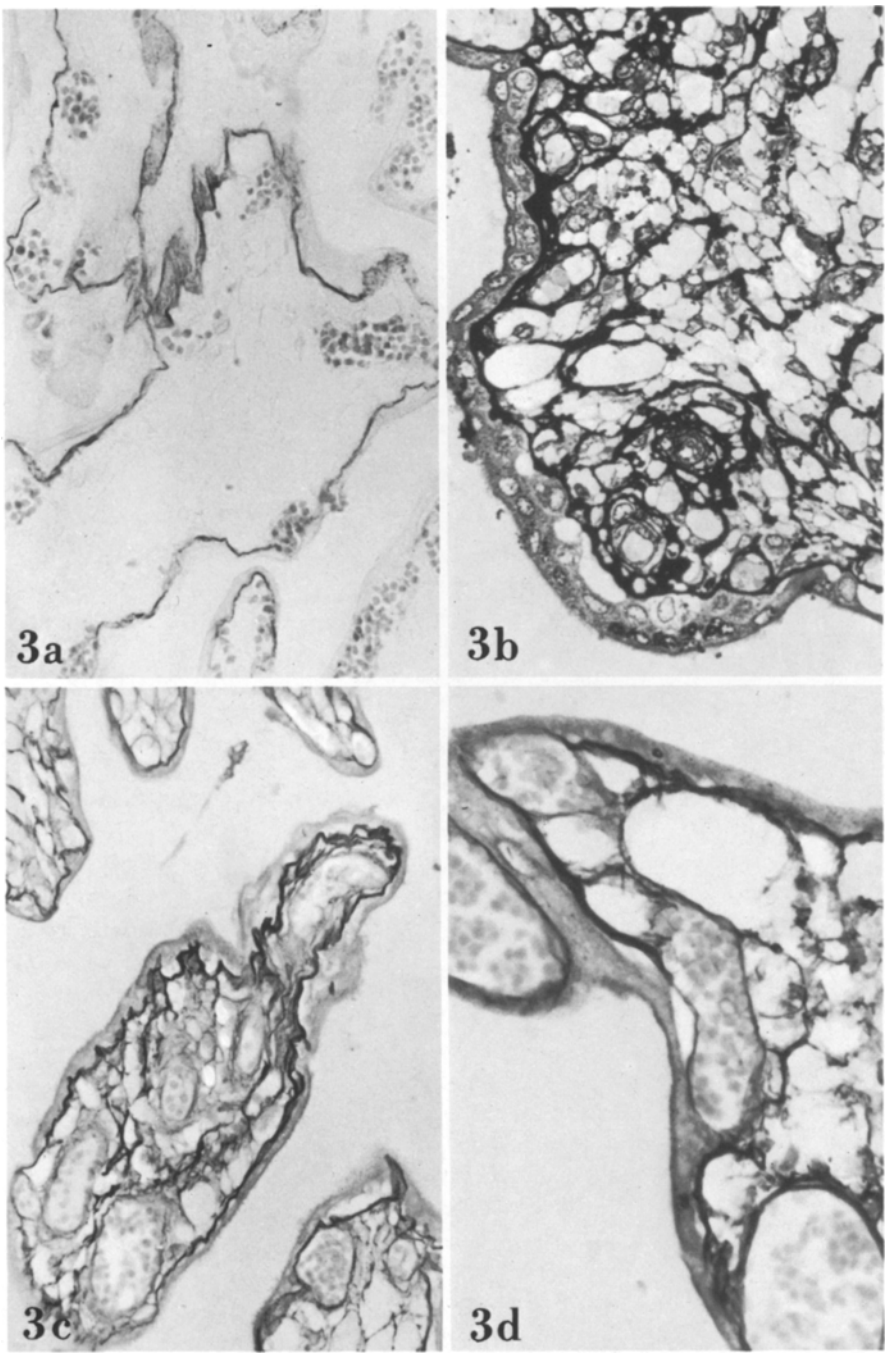

Fig. 3. **a** intense linear and granular positive reaction for haemosiderin in trophoblast basement membrane (Perls', ×360). **b** nodular condensations of trophoblast basement membrane and thickening in the stroma. Evident cytotrophoblast in the proximity of the nodularities (Periodic Acid Silver Methenamine, ×600). **c** diffuse splitting of basement membrane (Thioaldehyde Fuchsin, ×360). **d** markedly irregular thickenings of trophoblast basement membrane (Thioaldehyde Fuchsin, ×600)

subtrophoblastic iron deposits whereas in the thirty-six week old control placenta, single particulate positivity in the connective tissue of the villous stroma are observed.

After employing PAS and PASM in paraffin and in semi-thin sections the trophoblast and fetal capillary BMs present either a delicately positive or a uniformly thickened reaction. In many villi, focal condensations of material positive for the above two reactions are noted within the context of the BM and are particularly evident in proximity to a well-represented cytotrophoblast layer (Fig. 3b). PAS and PASM for all control placentae were positive at the trophoblast BM and stromal levels.

The TAF reaction, apart from the usual positivity of the elastic vessel walls, indicates an evident reaction at the level of the trophoblast BM (Figs. 3c, d).

The Van Kossa and Congo Red reactions are negative.

Direct immunofluorescence with anti-Ig G and anti-C_3 antibodies showed a slight positivity in the lumen of most blood vessels with absolute negativity in both the villous stroma and trophoblast.

Electron Microscopic Features

TEM observation of terminal villi reveals that the syncytio-trophoblast layer has an abundant, extensively dilatated granular endoplasmic reticulum, producing a diffusely vacuolated cytoplasmic aspect (Fig. 4). Moreover, the syncytiotrophoblast presents microvilli not only at the free surface, but, at the adjacent cellular and BM sites as well (Fig. 4). The nuclei present a moderately electron dense matrix with coarsely clumped chromatin and occasional prominent nucleoli. The perinuclear cistern is evident and nuclear pores are easily sean. Golgi areas are uncommon. Few mitochondria and osmiophilic granules are present.

The cytotrophoblastic cytoplasm is increased in volume with respect to the syncytio-trophoblast in many areas, appears structureless, is moderately electron dense and contains numerous free-floating polyribosomes.

The trophoblast BM is generally found to vary from 100–450 nm but, not infrequently, thickenings were observed which result from the splitting of BM-like material into a multiply folded pattern (Fig. 5a). The trophoblast BM contains numerous pleiomorphic dense bodies either isolated or in aggregates (Figs. 4, 5a). These bodies, not membrane bound, have a strongly osmiophilic core surrounded by filamentous, granular electron dense material (Fig. 5b). These bodies are constantly distributed in the trophoblast BM and at points of close contact between trophoblast BM and capillary BM.

The capillary endothelial cells are normal except in areas where they appear prominent because of a voluminous nucleus and an abundance of filamentous cytoplasmic material disposed in irregular bundles. Occasionally, sinusoidal capillaries present an irregular distribution of moderately electron dense BM-like material subendothelially (Fig. 6). Small fetal capillaries with two voluminous endothelial cells and corresponding BM, having potential lumina, were also observed (Fig. 7).

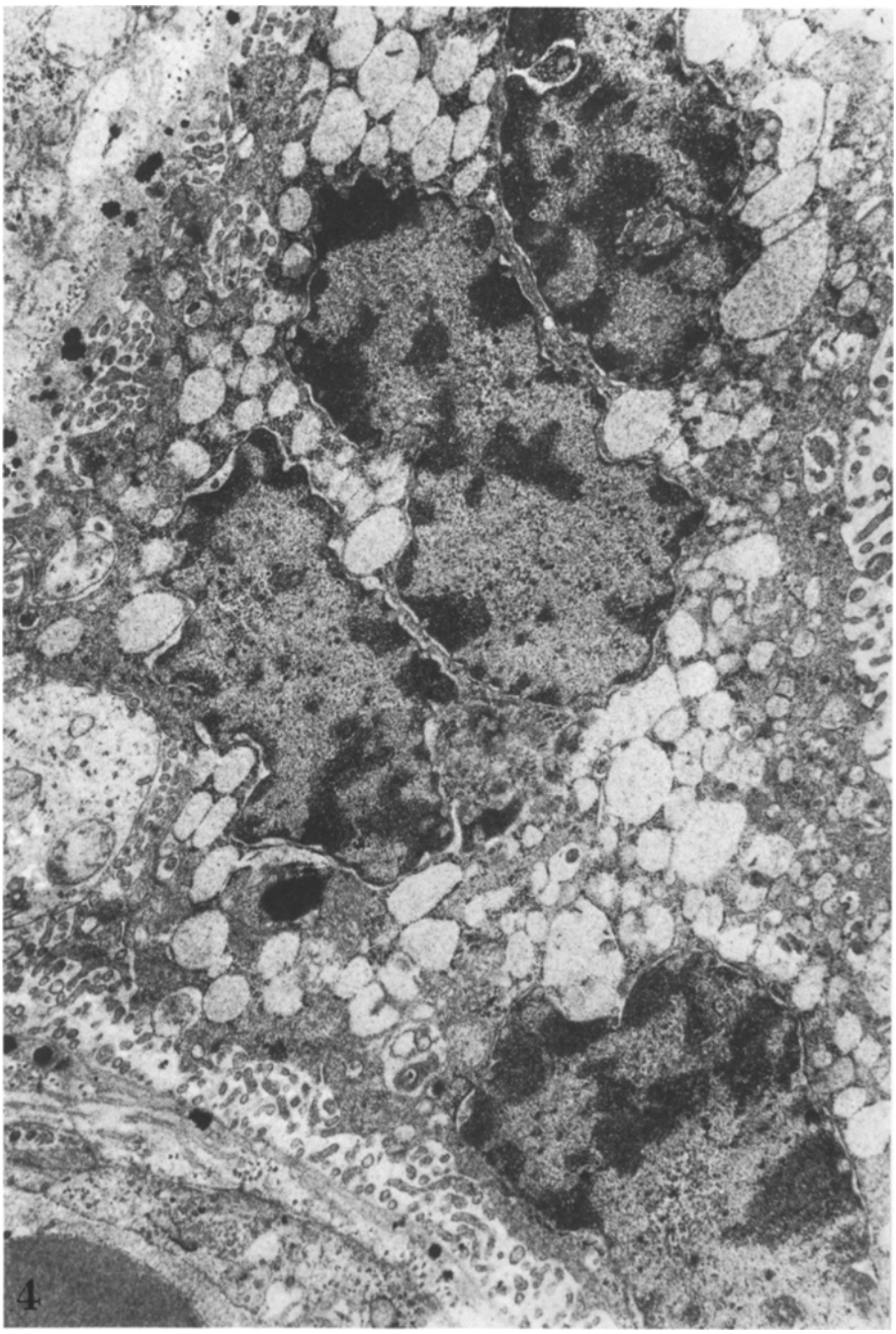

Fig. 4. A syncytial-trophoblast shows dense coarse clumps of marginated chromatin and vacuolization of granular endoplasmic reticulum. The fetal (*at right*) and basal surface (*upper and lower left*) demonstrate numerous microvilli and folds. Note the presence of isolated dense bodies in the basement membrane ($\times 30{,}000$)

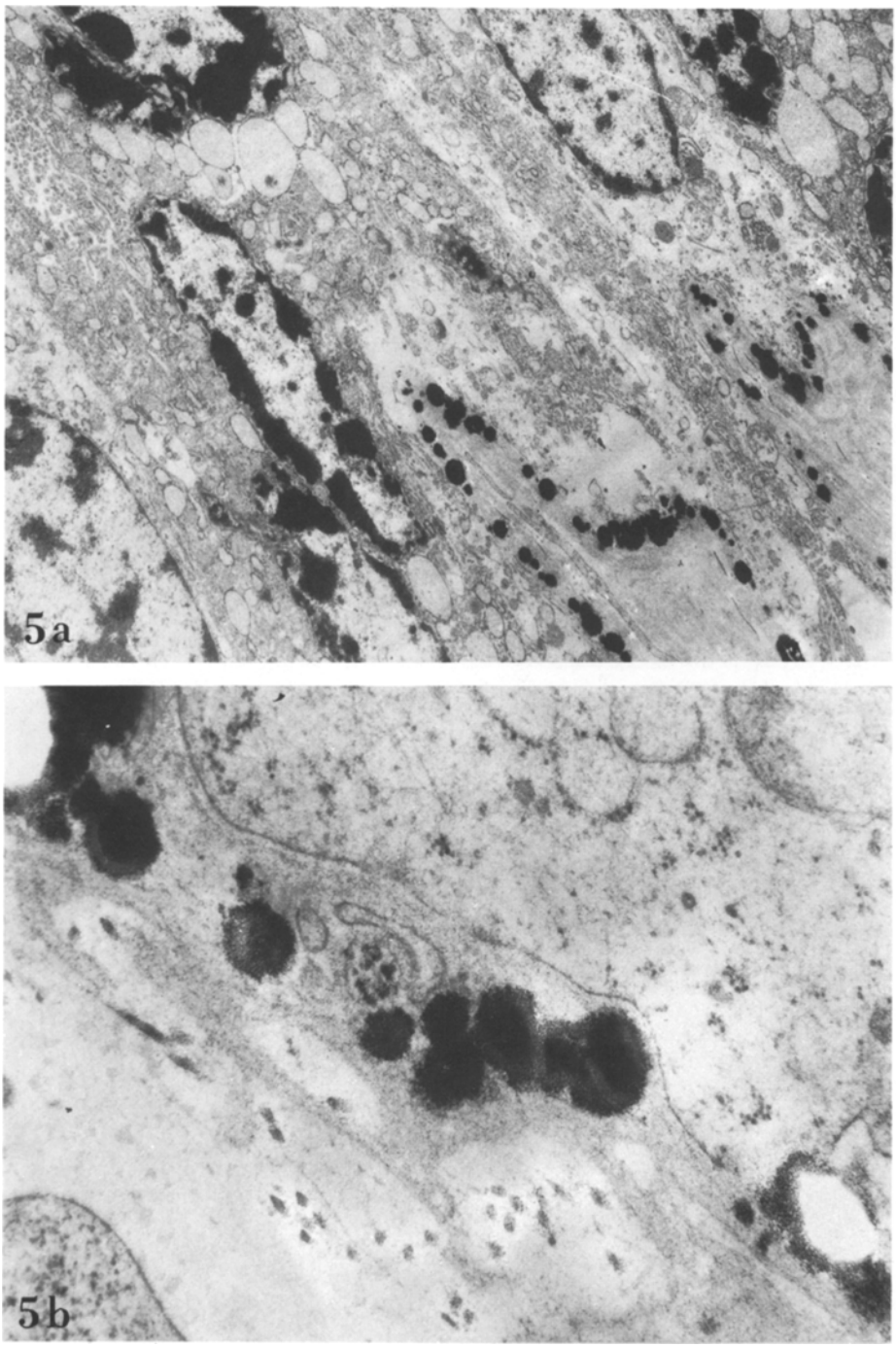

Fig. 5. a Multiply folded basement membrane constantly associated with numerous strongly osmiophilic bodies. At lower right, deposition of basement membrane like material. Portions of two large cytotrophoblast cells are seen (× 30,000). **b** Highly osmiophilic cores surrounded by gronulofilamentous components are noted in dense bodies. At the right a portion of a cytotrophoblastic element with free-floating polyribosomes is seen (× 70,000)

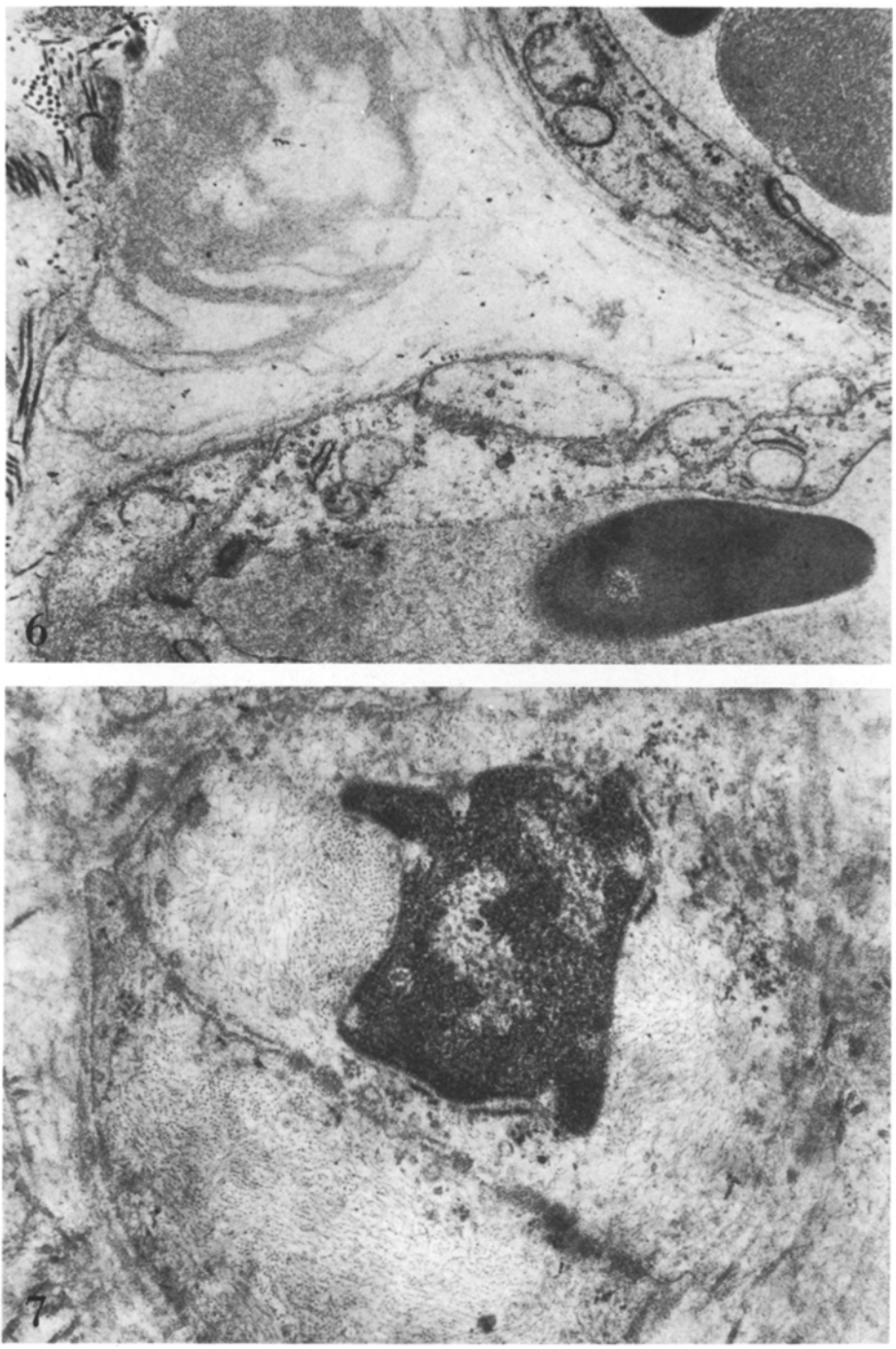

Fig. 6. Conspicuous lamination of basement membrane like material wedged between two endothelial cells ($\times 62{,}000$)

Fig. 7. A newly formed capillary: the endothelial cell cytoplasm is replete with filamentous aggregates ($\times 62{,}000$)

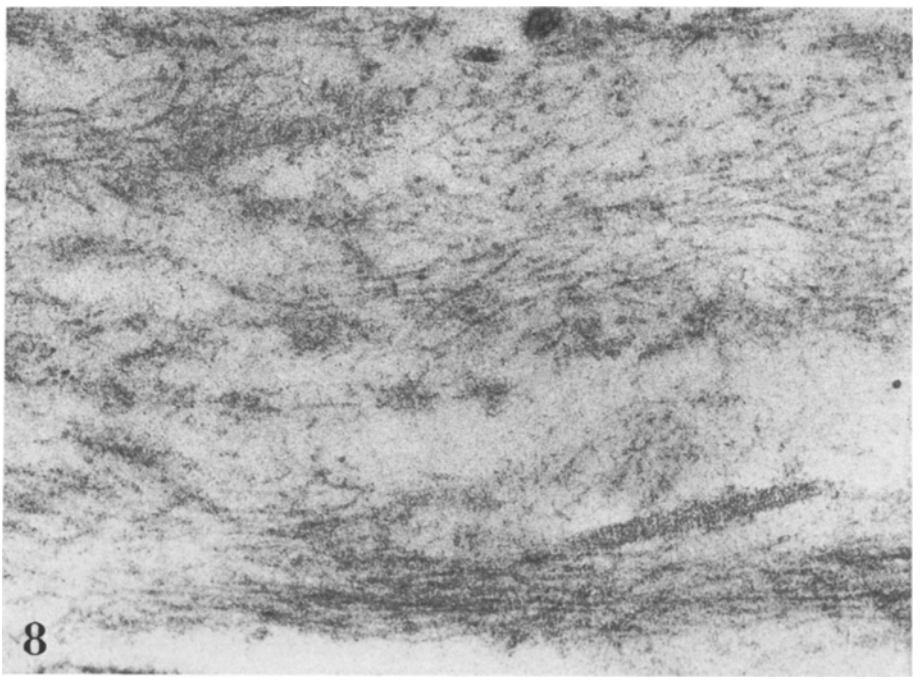

Fig. 8. In the villous stroma one observes the increased proportion of aperiodic microfibrils in relation to the collagen fibers present (× 80,000)

The terminal villous stroma shows large areas of amorphous material containing collagen fibers either isolated or in bundles which display evident periodicity. This matrix is rich in microfibrils which are granular in aspect and have no evident periodicity. In many areas, these filamentous units apparently converge with collagen fibers which display periodicity (Fig. 8).

Discussion

The few data regarding the CNF agree with the concept that there is an arrested maturation of the chorionic villi (Kouvalainen et al. 1962; Wiggelinhuizen et al. 1976; Kjessler et al. 1977). This maturation arrest is observed in early gestational periods (Wiggelinhuizen et al. 1976; Kjessler et al. 1977) but is most pronounced in the findings of Kouvalainen et al. (1962) whose work represents the only analysis to date of a full-term placenta.

Our findings are concordant with the concept of arrested villous maturation as reported previously. This placenta is observed at thirty-six weeks gestation but has the morphological aspect compatible with that of one from the first-second trimester. In fact, it is largely composed of persistent embrionic villi with marked stromal oedema and numerous Hofbauer cells. An interesting ultrastructural finding in the stroma of the embrionic villi is the increased number of aperiodic microfibrils.

These microfibrillar areas, PASM positive, appear to be reticulin in nature. During normal placental maturation these areas would have been replaced, for the most part, by collagen fibers. These morphological findings are similar to Liebhart's observations (1974) in diabetes mellitus and are suggestive of deficient stromal maturation, presumably accompanied by permeability alterations. In our case, enhanced water retention – manifesting ultrastructurally as empty spaces and histologically as oedematous villi rich in Hofbauer cells – results in loosening of the connective structure.

The embryonic villi are insufficiently vascularized but, on the other hand, capillary hyperplasia of the sinusoidal type has been observed in many villi. Capillary proliferation is also documented electron microscopically by the presence of immature capillaries. These latter represent an anomalous finding in a thirty-six week old placenta. Moreover, in capillary walls, focal laminations of homogeneous moderately electron dense BM-like material are observed subendothelially. This material is PASM positive and could represent the morphological substrate for the progressive development of the sinusoidal dilatations observed.

These vascular alterations occur in the reticular stroma of swollen villi resembling the morphological aspects of chorionangiomatosis. Chorionangiomatosis is not noted in any of the previous work concerning the placenta in CNF, even though a placenta with multiple chorionangiomata has been reported in a new kind of glomerulopathy causing CNS (Fischbach et al. 1978).

Persistent embryonic villi and chorionangiomatosis are recognized as primary disorders of placentation (Höpker and Ohlendorf 1979). Their presence in this case of CNF indicates that they arise primitively during the first and second trimesters of pregnancy.

Our findings also document evident alterations of the trophoblast BM. The discrete positivity with AB pH 1 and the very tenuous positivity with AB pH 2.5 observed in this case of CNF and in control placentae of three months gestational age, indicates the presence of sulphated acid mucopolysaccharides and documents trophoblast BM immaturity. The latter condition is also revealed by the peculiar distribution of haemosiderin along the trophoblast BM as documented by Perls' reaction and the presence of osmiophilic granulo-filamentous bodies. This finding, not previously described in CNF, is common to the second trimester of normal pregnancy, whereas, in the third trimester is only sometimes observed in the villous stroma and never in the BM (McKay et al. 1958).

Another alteration of the trophoblast BM concerns focal, apparently nodular, thickenings documented by PASM. When TAF is employed these thickenings are even more distinct. TAF positivity is probably due to the rich quantity of disulfide bonds which are known to be localized in the collagen and non-collagen components of the BM as reported in the review by Kefalides et al. (1979). These thickenings upon TEM observation are constituted of BM-like material, are almost always located near voluminous cytotrophoblast cells and may indicate a possible biosynthetic participation of these cells, as Fox (1978a) suggests occurs in all states of cytotrophoblastic proliferation.

Trophoblast BM thickening is encountered in other pathological conditions e.g. cases of intrauterine hypoxia, low birth weight neonates, pre-eclampsia,

diabetes mellitus and in some cases of maternal-fetal Rh incompatibility (Fox 1978b). The aetiology and pathogenesis of these thickenings, even though frequently attributed to hypoxia, remain unknown. It is to be pointed out that in our placenta, hypoxic phenomena are probably not a primary determinant of BM thickening in as much as VSMs are increased in number and no infarcted areas are observed. Moreover, the trophoblastic BM thickening, described in this heterogeneous group, is referable to modest variations of BM caliber rather than to nodular condensations as seen by us in the CNF.

The nodular thickenings noted by us in this placenta may instead indicate a primitive membrane alteration. It has been generally assumed that morphological alterations of BMs in human and experimental disease must be accompanied by associated changes in their chemical composition and structure (Kefalides et al. 1979). Mahieu et al. (1976), in analyzing renal glomerular BMs from three patients with CNS of which one patient was similar to Finnish type, noted an increase in the amounts of 3- and 4-hydroxyproline, hydroxylysine and glucosylgalactosylhydroxylysine. These same authors documented focal thickenings of renal glomerular BMs emploing the PASM reaction. Instead, in three proven cases of CNF, Tryggvason (1977) found a decrease of these three amino acids, a decrease in glycine, and an increase in lysine, arginine and alanine.

Our findings, at the level of the placental villous BM and stroma, may possibly represent the morphological equivalent of these biochemical alterations. One may speculate that BM and BM-like material are resistant to lysis and phagocytosis and thereby remain intact as if in the early stages of embryonic development. This may result either because an anomalous structural arrangement renders these membranes resistant to enzymatic activity of the surrounding microenvironments, or because connective tissue cells (macrophages, fibroblasts) are functionally immature and consequently fail to elaborate sufficient quantities of those enzymes involved in collagen degradation.

In this case of CNF, in view of negative findings in support of inflammatory, ischaemic or immunopathological processes, and the absence of appreciable endocrine disorders, we consider that the only significant possibility remaining is that placentomegaly is related to deficient collagen degradation resulting in the primitive membranopathy seen.

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