THE SUBPLACENTA OF THE GUINEA PIG: AN ELECTRON MICROSCOPIC STUDY

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The development, histology and histochemistry of the guinea-pig subplacenta have been described in a previous paper in this *Journal* (Davies, Dempsey & Amoroso 1961). The subplacenta is a specialized chorionic zone derived by proliferation and infolding of the foetal ectoderm in the floor of the 'central excavation' or mesodermal core of the chorio-allantoic placenta. It consists of cytotrophoblastic lamellae enclosing chorio-allantoic mesenchyme on their foetal surface and giving rise to syncytial trophoblast on their maternal surface. The syncytium of the subplacenta differs from that of the chorio-allantoic placenta in that it is vascularized exclusively by foetal vessels and contains, instead of maternal blood channels, a system of lacunar spaces filled with amorphous protein-like material. This material gives a strong periodic acid-Schiff (PAS) reaction after the removal of glycogen by saliva and is probably glycoprotein in nature. In addition, the syncytial cytoplasm shows a delicate PAS-positive stippling, indicating the presence of granules or droplets just within the resolving power of the light microscope, and also contains a large amount of glycogen. The subplacental syncytium is prolonged into the basal decidua in the walls of the maternal placental vessels, the endothelium of these vessels being replaced by trophoblastic cells at an early stage. Due to some doubt concerning the cytotrophoblastic or syncytial nature of the vessel wall in such areas the term 'endotrophoblast' was used. Arguments were put forward for a possible gonadotrophic function of the subplacenta or, alternatively, for its possible involvement in the absorption of substances of high molecular weight from the decidua and their transport to the foetal circulation.

The electron-microscopic observations in this paper have clarified many of the points in the previous paper which could not be answered owing to the inherent limitations of the light microscope. Most especially they have revealed the true nature of the lacunar spaces and the relationship of these to the intercellular spaces of cytotrophoblastic layer, as well as the fine structure of the syncytial trophoblast and its 'endotrophoblastic' extensions into the walls of the decidual vessels.

MATERIAL AND METHODS

Specimens were obtained from pregnant guinea-pigs at various stages of gestation from about the 20th day to term (68 days). Portions of the placenta were fixed by immersion in the osmium tetroxide mixtures recommended either by Palade (1952) or Dalton & Felix (1955). Adequate fixation was obtained after 1 hr. Tissues were

* Supported in part by a grant RG-3784 from the National Institutes of Health, United States Public Health Service, and in part by a grant from the Josiah Macy Jr. Foundation.

then dehydrated and embedded in a mixture of methyl- and butyl-methacrylate in a ratio of 1:6 or 1:8. Sections were cut on a Porter–Blum microtome and, after orientation by the use of the phase-contrast microscope, were examined in the electron microscope (RCA model EMU 2E).

RESULTS

The following observations with the electron microscope cover a wide range of placental areas in the guinea-pig placenta, viz, the subplacenta, the 'coarse' and 'fine' syncytium of the chorio-allantoic labyrinth, the parietal endoderm and its underlying layer of chorionic giant cells, the modified walls of the major placental vessels at various levels, and the decidual cells. The interrelationship of these parts has been illustrated diagrammatically in a previous paper (Davies, *et al.* 1961). Many of these observations are of a limited nature, the subplacenta and vessel walls alone being considered in detail. However, a survey of such a wide range of placental structures has been justified on the grounds that they form an integrated whole in terms of development and probably also of ultimate physiological function.

Terminological note

The term 'endoplasmic reticulum' is used to describe the vesicular and membranous structures of indeterminate origin within the cytoplasm. The term 'ergastoplasm' is used only when these membranous and vesicular elements of the endoplasmic reticulum are associated with small granules (Palade, 1952). Such cytoplasmic areas are generally held to show marked cytoplasmic basophilia after staining with the basic aniline dyes.

Subplacenta

The subplacenta at the 25th day is illustrated as seen in the phase-contrast microscope (Pl. 1, fig. 1) and at a low magnification in the electron microscope (Pl. 2, fig. 2). The cytotrophoblastic layer consists of a two or three-layered epithelium resting on a thin basement membrane which separates it from the foetal mesenchyme. There are indications that the epithelium is pseudo-stratified and that all the cytotrophoblastic cells may reach the basement membrane at some point. The adjacent walls of the cells are moulded by mutual pressures. For the most part the walls are in contact, the apposing cell membranes being modified in some areas by the development of terminal bars. Between the areas of apposition the intercellular spaces are irregularly dilated, especially at the angles between two or more cells. The presence of minute protrusions of the cell membrane or microvilli into the spaces suggests that they are not shrinkage artifacts but that they may exist in the living state. The nuclei of the cytotrophoblastic cells are large relative to the volume of the cytoplasm and contain several prominent skein-like nucleoli. The cytoplasm is finely granular and contains a few mitochondria but no fat or other inclusions. These cells, which show marked cytoplasmic basophilia in the light microscope, contain many of the dense particles which have been correlated with such basophilia in other sites (see Palade, 1952). The syncytial trophoblast, which is formed by proliferation from the cytotrophoblast, is limited externally by a definite cell membrane where it faces the cytotrophoblastic layer. At the interface between

syncytial and cytotrophoblastic elements of the subplacenta, several situations may exist. The plasma membranes of the two layers may lie in simple apposition, being thickened in some areas by the development of terminal bars. In other places the two layers are separated producing an extracellular cleft lined by microvilli which is continuous with the dilated intercellular spaces of the cytotrophoblastic layer. The cytoplasm of the syncytial trophoblast is coarsely granular and contains a few mitochondria which are smaller than those of the cytotrophoblastic cells and are also less dilated. Patches of flocculent material probably represent deposits of glycogen. The large vacuoles or lacunae observed in the subplacental syncytium (Pl. 1, fig. 1) appear in the electron microscope as irregular spaces lined by microvilli and occupied by a small amount of coagulated material. In the early stages of pregnancy (Pl. 2, fig. 2) many of the microvilli are swollen, suggesting a hydropic change, perhaps associated with an active transfer of water into or out of the lacunae.

The changes in the subplacenta with advancing gestation are shown in Pl. 3, fig. 3. The cytotrophoblastic layer is reduced to a single row of cells though there are many regions where this layer remains multilaminar until at least the 50th day. The cytotrophoblast is absent in some areas allowing the syncytium to rest directly on the basement membrane. In these areas there is a clearly defined space between the syntium and the basement membrane. Delicate microvilli, protruding from the plasma membrane of the syncytium, project into the space and some are implanted on the basement membrane by small, foot-like processes. Elsewhere, along the boundary between the syncytium and the cytotrophoblastic layer, the syncytium is less intimately applied to the cellular layer than was the case in the earlier stages and is separated from it by a cleft-like space into which project microvilli derived from the plasma membrane of the syncytium. The true syncytial character of the subplacental syncytium is also shown in Pl. 3, fig. 3. The lacunar spaces are lined by microvilli similar to those at the interface between the syncytium and the cytotrophoblastic layer.

Pl. 4, fig. 4, illustrates the details of the subplacental syncytium at the middle of gestation. The syncytial mass is traversed by a system of vacuoles or lacunae. Many of the lacunae are dilated and lined by delicate microvilli, the contained material being sparse and of moderate electron density. In other areas the lacunae are narrowed or partially collapsed. In these the microvilli are absent or poorly developed and the contained material is of higher electron density. It is reasonable to suppose that the occurrence of the most electron dense material in association with an absence or reduction in the number of microvilli indicates stagnation of the lacunar contents. The syncytial cytoplasm contains a few scattered mitochondria. There are large areas of the cytoplasm occupied by featureless masses of granular material of low electron density, probably representing glycogen in which the subplacental syncytium is particularly rich (Davies, *et al.* 1961). Isolated islands of ergastoplasm are also found. The ergastoplasmic membranes are arranged as parallel tubules which contain amorphous material resembling in over-all electron density the material within the syncytial lacunae. The syncytial cytoplasm also contains many granules or droplets of marked electron density. These droplets are just within the limits of resolution of the light microscope and may correspond with the stippled areas of PAS positive material previously described in the subplacental

syncytium (Davies, *et al.* 1961). They show no predilection for any part of the cytoplasm, being found as often in the vicinity of the nucleus as in the neighbourhood of a lacunar space.

The junction between the subplacental syncytium and the cytotrophoblast is illustrated at higher magnification in Pl. 5, fig. 5. The plasma membrane of the syncytial trophoblast is in smooth apposition with the surface of the cytotrophoblastic cells but shows localized dilations lined by microvilli and continuous with the intercellular spaces of the cytotrophoblastic layer. Vacuoles found within the marginal cytoplasm of the syncytium and the cytotrophoblastic cells may be indicative of pinocytosis of fluid from the extracellular spaces.

The relationship of the syncytium to the basement membrane and foetal mesenchyme at the 54th day is shown in Pl. 6, fig. 6. The disappearance of the cytotrophoblast in many areas is interpreted as a senescent change, presumably associated with the exhaustion of this germinal layer in the continued production of syncytium. Where the cytotrophoblastic layer is deficient the syncytium again presents the characteristic pattern of microvilli, many of which are implanted on to the basement membrane by slightly expanded processes (Pl. 7, fig. 8). The basement membrane of the trophoblast is thickened and fibrillar in the later stages of pregnancies, and the foetal mesenchyme contains fewer cellular elements and a considerable amount of collagen. The confluence of the lacunae into the characteristic lattice-like pattern so well revealed in periodic acid-Schiff preparations is illustrated in Pl. 6, fig. 7: the lacunar material appears inspissated and embedded in the microvilli lining the walls of the cavity.

Maternal placental vessels

Beginning about the 15th day the subplacental trophoblast invades the decidual tissues along the path of the capillaries, some of which are later modified as the major placental vessels. The endothelium is eroded and replaced by trophoblastic epithelium. The term 'endotrophoblast' has been used to describe this lining epithelium since its syncytial or cytotrophoblastic character could not be determined with the light microscope. The nature of the endotrophoblast has been clarified by the use of the electron microscope. The cellular constitution of the limiting walls of the maternal blood channels within the placenta and the subplacenta and also of the major placental vessels during their course through the decidua has been traced at all levels and is illustrated by representative sections.

(1) *Within the chorio-allantoic placenta:*

The foetal and maternal blood come into closest apposition in the areas of so-called 'fine syncytium'. Here the maternal blood channels of the chorio-allantoic placenta are lined by endotrophoblast (Pl. 7, fig. 9), which appears to consist not of a true syncytium but of overlapping sheets of trophoblastic cells (Pl. 7, fig. 9). Cytotrophoblastic cells are identifiable in the chorio-allantoic placenta as late as the 25th day. They are always separated from the maternal blood stream by a layer of syncytial trophoblast. In the areas of 'coarse syncytium', which are not vascularized by foetal vessels, the fine structure of the lining of the maternal blood spaces resembles syncytial trophoblast (Pl. 7, fig. 10). However, the presence of ill-defined

membranes of considerable electron density within the endotrophoblastic wall suggests that the latter may also consist of overlapping sheets of cytoplasm and is, therefore, cytotrophoblastic rather than syncytial. The problem requires further study, however.

Small, extremely electron dense bodies are observed among the microvilli of the endotrophoblastic lining of the maternal blood spaces during the first half of pregnancy, especially in the transitional zone between the chorio-allantoic placenta and the subplacental syncytium (Pl. 7, figs. 9, 10). These bodies may have an angular profile, suggesting that they may have a crystalline structure. They are visible in the phase-contrast microscope as a delicate stippling of faintly osmiophilic material.

(2) *Within the subplacenta*

The large maternal placental vessels traversing the subplacenta near its edge continue to be lined by endotrophoblast which is syncytial in type (Pl. 8, fig. 11). Since these vessels are formed by the union of blood channels emerging from the base of the lobules of the chorio-allantoic placenta, it is likely that they are venous in character. The endotrophoblast appears vacuolated due to the presence of swollen mitochondria and dilated sacs and channels of the endoplasmic reticulum. Many of the latter contain a coagulum of amorphous material of moderate electron density. Residual cytotrophoblastic cells are found in relation to the basement membrane of these vessels until at least the 35th day of pregnancy. The endotrophoblast may rest on a basement membrane which separates it from the foetal (chorio-allantoic) mesenchyme (Pl. 8, fig. 11), or may merge insensibly with the subplacental syncytium with no intervening basement membrane (Pl. 8, fig. 12). The lacunae in immediate relationship to the maternal vessels are larger than in the rest of the subplacenta. They are separated by extremely attenuated septa of syncytial trophoblast and also contain amorphous material of varying electron density. This material, like that within the other subplacental lacunae, is strongly PAS positive. Large masses of PAS positive material within the outer portion of the walls of the large maternal veins piercing the subplacenta were described previously (Davies, *et al.* 1961): their relationship to the cellular components of the vascular wall could not be determined, however.

(3) *Within the necrotic zone of the basal decidua*

The maternal vessels within this zone of the basal decidua show striking modifications of their walls which consist of varying combinations of endotrophoblast with maternal endothelium. Four general types of vessels have been observed in which the walls consist of: (1) syncytial trophoblast alone, (2) maternal endothelium and syncytial trophoblast, (3) maternal endothelium and giant cytotrophoblastic cells, and (4) maternal endothelium alone.

The syncytial lining of the first type of vessel (Pl. 9, fig. 13) resembles in general features the endotrophoblast of the vessels at the level of the subplacenta. The vacuolation of the cytoplasm is very variable from one vessel to another. The microvilli at the luminal edge are branched and frequently appear swollen and hydropic. The cytoplasm contains ergastoplasm, swollen mitochondria and aggregates of

electron dense granules similar to those of the ergastoplasm in other sites. An irregularly thickened basement membrane intervenes between the endotrophoblast and the necrotic decidua. The latter is traversed by collagen fibres and contains amorphous masses of material which are also PAS positive.

The epithelium in immediate contact with the maternal blood in the second type of decidual vessel is the maternal endothelium (Pl. 9, fig. 14). The endothelial cells are imbricated and the plasma membranes show localized thickenings or terminal bars. The cytoplasm contains lipid inclusions. A thin basement membrane separates the endothelium from the endotrophoblast, and a second basement membrane separates the endotrophoblast from the necrotic decidual tissues. The syncytial cytoplasm resembles that of the large maternal vessels at the level of the subplacenta (compare Pl. 8, fig. 11). The mitochondria are swollen and are distinguishable from the all-pervading sacs and channels of the endoplasmic reticulum by their thicker walls and by remnants of the cristae. In other vessels of the same type the endotrophoblast is less vacuolated (Pl. 10, fig. 15). The syncytium is implanted on the endothelial basement membrane by cytoplasmic processes or 'feet' (Pl. 9, fig. 14; Pl. 10, fig. 15), reminiscent of the podocytes of the visceral layer of Bowman's capsule in the renal glomerulus. The foot-processes enclose a labyrinthine subendothelial space which is continuous in many areas with the complex system of dilated sacs and channels within the endotrophoblastic cytoplasm. The transition from a vessel of the second type to one lined only by maternal endothelium (fourth type) is abrupt and at this point the two basement membranes become confluent (Pl. 10, fig. 16). The maternal vessels deeper within the basal decidua are generally composed solely of maternal endothelium resting on a thin basement membrane into which are inserted collagen fibres (Pl. 10, fig. 17).

In vessels of the third type (Pl. 11, fig. 19) the maternal endothelium is thickened. The endothelial cytoplasm is finely granular and contains mitochondria which may be small and electron dense with well-marked cristae or may be greatly swollen. The proximity of cells containing both types of mitochondria suggests that the swollen character of the organelles in some cells is not due to faulty fixation. Endothelial cells with swollen mitochondria also tend to have large cytoplasmic vacuoles as well as smaller vacuoles within the marginal cytoplasm adjacent to the maternal blood. The basal plasma membranes of the endothelial cells are highly irregular and rest on a very attenuated and apparently discontinuous basement membrane. External to the endothelium is a thick endotrophoblastic layer composed of large mononuclear cytotrophoblastic cells. They have been interpreted as trophoblastic since their fine structure clearly distinguishes them from decidual cells (see Pl. 12, fig. 21). It has not been possible to determine if the giant cytotrophoblastic cells form a single layer having limited areas of contact with the endothelial basement or if they are stratified. They are implanted on to the endothelial basement membrane by delicate foot processes, the terminal portions of which are expanded and rest in depressions hollowed out in the plasma membranes of the endothelial cells, the basement membrane being very attenuated or indistinguishable at the areas of contact. The spaces between the cytotrophoblastic cells are dilated and incompletely lined by microvilli. These spaces are, in turn, continuous with the complex subendothelial space enclosed by the branching processes of the cytotrophoblastic cells. Wisps of

coagulated material of moderate electron density are found within the intercellular spaces and the subendothelial space. The giant cytotrophoblastic cells are of two types, dark and light cells. The cytoplasm of the dark cells is electron dense and contains closely packed membranous and granular elements of the endoplasmic reticulum. The light cells have a cytoplasm of lower electron density which contains more widely scattered ergastoplasmic membranes and granules and many large vacuoles containing amorphous material. Smaller vacuoles are found within the marginal cytoplasm bordering the dilated intracellular spaces. A re-examination of the vessels of the basal decidua in histological preparations stained with haematoxylin and eosin showed that vessels of the third type may easily be recognized. The large cytotrophoblastic cells are visible as rounded vesicular cells in the outer part of the wall.

(4) *Within the residual zone of the basal decidua*

A residual layer of modified decidual cells separates the necrotic zone from the myometrium and persists until the end of pregnancy. Maternal placental vessels passing through this zone are for the most part lined only by maternal endothelium (fourth type; Pl. 10, fig. 18) or, more rarely, by endotrophoblast of the syncytial type (first type; Pl. 12, fig. 20). The endothelium or syncytium rests on a basement membrane by which it is separated from the decidual tissues. There is a wide subendothelial space containing wisps of collagen and masses of amorphous material. The latter is continuous with material of similar electron density within the complex infoldings of the surface plasma membranes of the decidual cells (Pl. 10, fig. 18). The plasma membranes of adjacent decidual cells are sinuous and interlock in a complex manner. The cells are separated by a substance of marked electron density which also extends into the bays resulting from the inflexion of the plasma membranes into the marginal cytoplasm (Pl. 12, fig. 21). The bays in some instances may be traced into continuity with large intracellular vacuoles lined by microvilli and containing amorphous material of lower electron density than that between the cells. These intra-cytoplasmic vacuoles may be very large, dwarfing the nucleus and may correspond with the large acidophilic and PAS positive masses observed by light microscopy in the giant decidual cells of the junctional region.

Present studies have not permitted a correlation between the varied cellular composition of the walls of the decidual vessels with their arterial or venous character. The differences in level at which the maternal endothelium reappears as the vessels are traced toward the myometrium probably reflect the extent of invasion of the decidua by the subplacental trophoblast. Moreover, the replacement of the endothelium of these vessels by trophoblastic elements certainly involves both arterial and venous channels. Careful injections of the vessels will have to be carried out before the problem can be solved satisfactorily.

Endoderm of the parietal wall of yolk sac and chorionic giant cells

The endodermal cells comprising the parietal wall of the inverted yolk sac and the underlying layer of chorionic giant cells are well developed and show an intimate morphological association throughout most of gestation. The cells of the parietal endoderm form a pseudostratified columnar epithelium having irregularly dilated intercellular spaces which confer upon this layer a characteristic tufted appearance

in the light microscope. The plasma membranes of the cells which face the decidual cavity are thrown up into long slender microvilli (Pl. 13, fig. 22). The decidual cavity contains a flocculum of precipitated material. The plasma membranes bordering on the dilated intercellular spaces are inflected into the cytoplasm to form a marginal system of recesses and bays containing amorphous material similar to that which fills the intercellular spaces. The plasma membranes of adjoining cells are thickened, forming attachment plates or terminal bars. The cytoplasm of the endodermal cells contains granular and membranous elements of the endoplasmic reticulum though these cells are not basophilic when studied in the light microscope. The mitochondria are small and scattered and the cytoplasm contains no lipid or other inclusions.

The chorionic giant cells are mononuclear cytotrophoblastic cells showing distinct limiting plasma membranes which interlock in a complicated manner (Pl. 13, fig. 22). The cytoplasm contains a few scattered mitochondria, islands of ergastoplasm which are predominantly perinuclear in position, large vacuoles and many homogenous inclusions of varying electron density. The vacuoles occur mainly near the plasma membrane, and in many cases are clearly derived by inflexion of the membrane. They contain flocculent material and many droplets, some of which are extremely electron dense. The homogenous inclusions are bounded externally by definite membranes but show no recognizable internal structure to link them with the mitochondria.

Reichert's membrane occupies the broad interval between the endodermal cells and the chorionic giant cells (Pl. 13, fig. 22). It is faintly fibrillar with no identifiable collagen. The substance of Reichert's membrane is prolonged into the dilated intercellular spaces of the endodermal layer, these extensions of PAS positive material being clearly visible in the light microscope (Davies *et al.* 1961). It is also continuous with the amorphous material contained within the superficial vacuoles of the cytoplasm of the chorionic giant cells.

DISCUSSION

The salient histological features of the subplacenta of the guinea-pig have been confirmed by the use of the electron microscope. The syncytial vacuoles or lacunae, the true nature of which could not be resolved in the light microscope, have been shown to be well differentiated spaces lined by microvilli. The lacunae arise as apparently isolated spaces within the subplacental syncytium in the early stages of pregnancy but communicate extensively in the later stages. They must probably be regarded as intracytoplasmic vacuoles rather than as modified extracellular spaces. Their true nature and that of the marginal extracellular space between the subplacental syncytium and the cytotrophoblast is, however, bound up with the difficult problem of the origin of the syncytium from the parent cytotrophoblastic layer. That protoplasmic sheets, essentially cytotrophoblastic in nature and separated by true extracellular spaces, can occur in the placenta is shown by the observations of Wislocki & Dempsey (1955*b*) on the lining epithelium of the maternal blood spaces in the chorio-allantoic placenta of the rat which consists of imbricated mononuclear or occasional binuclear cells. Preliminary observations reported in this paper suggest that the same may be true of the chorio-allantoic placenta of the guinea pig. In the light of these observations, the presence of the lacunae within the subplacenta

may reflect some peculiarity in the origin of the syncytium which deserves more careful study with the electron microscope.

The material within the lacunae corresponds with the PAS positive masses previously identified within the subplacental syncytium. The origin of this material is entirely speculative. Possible precursors of the material may exist in the form of the electron dense droplets or within the dilated ergastoplasmic sacs of the syncytial cytoplasm. The subplacental syncytium is also characterized by the paucity of mitochondria and the presence of large amounts of glycogen. The latter observation is a further example of the generalization of Wislocki, Deane & Dempsey (1946) that glycogen is abundant in parts of the placenta which are far removed from a source of blood supply and may therefore be characterized by a high rate of anaerobic glycolysis. Lipid is absent from the subplacental syncytium, suggesting that it is not concerned in the elaboration or storage of the steroid hormones. The cytotrophoblastic cells which give rise to the syncytium are characterized by their large nuclei, multiple skein-like nucleoli and their granular cytoplasm. These features may possibly be correlated with an active synthesis of ribonucleoprotein by these cells which are strongly basophilic after staining with the basic aniline dyes.

The fine structure of the syncytial trophoblast (endotrophoblast) which is in contact with the maternal blood entering either within the chorio-allantoic placenta or within the walls of the maternal placental vessels differs strikingly from that of the subplacental syncytium. The microvilli at the luminal surface, the swollen mitochondria, the dilated endoplasmic reticulum and the absence of glycogen are features of the endotrophoblast which mark it as an actively absorbing epithelium. It resembles the syncytial trophoblast on the surface of the human chorionic villi, as described in the electron microscope by Boyd & Hughes (1954) and by Wislocki & Dempsey (1955*a*).

Extensions of the subplacental trophoblast into the walls of the decidual vessels have been described in this paper and present a variety of interesting relationships between the endotrophoblast and the maternal blood. In the vessels immediately beneath the subplacenta, which are lined only by syncytial trophoblast, the relationship may be termed haemochorial as in the chorio-allantoic placenta. Deeper within the decidua the maternal endothelium is interposed between the endotrophoblast and the maternal blood stream, thus representing a haemoendothelial condition. In some cases the endotrophoblast is syncytial and in others is composed of giant cytotrophoblastic cells. In both cases the endotrophoblast is implanted on the endothelial basement membrane by cytoplasmic feet and, especially in the former case, strikingly resembles the conditions in the chorio-allantoic labyrinth of the cat (Dempsey & Wislocki, 1956). Complex modifications of the surface plasma membranes and swelling of the motochondria of the endotrophoblast have been described in this paper and may be tentatively correlated with the occurrence of fluid exchanges between the maternal and foetal organisms, with or without the intervention of the maternal endothelium. The walls of the decidual vessels remain very simple in relation to the residual zone of the basal decidua and consist of maternal endothelium alone or, more rarely, of syncytial trophoblast. The relationship of these vessels to the decidual cells, with a minimum of intervening mural elements, suggests that they may mediate important exchanges of materials between the maternal blood

and the decidual tissues. The electron dense material which encapsulates individual decidual cells, and which is strongly PAS positive in the light microscope, also extends into the marginal bays and intracellular vacuoles of the cells, suggesting that it may be elaborated by them. The encapsulating material also extends into the subendothelial space around the large decidual vessels. The replacement of the endothelium of the large maternal placental vessels within the decidua by large cells of unknown though presumably trophoblastic origin has been described in many rodents (Duval, 1890; Mossman, 1937). These vessels have not, however, so far been studied with the electron microscope.

The fine structure of the endodermal cells of the parietal walls of the inverted yolk sac and of the underlying layer of chorionic giant cells are consistent with the view that these layers are involved in the absorption of materials from the decidual cavity. The passage of such materials may be modified by the presence of Reichert's membrane between the two layers. Evidence that this membrane may arise as product of the chorionic giant cells has been presented. The membrane in the guinea-pig differs from that of the rat is being less compact and less fibrillar (Wislocki & Dempsey, 1955*b*). It also differs from visceral walls of the yolk sac which is fenestrated, more coarsely fibrillar and receives the terminations of collagen and reticular fibres reaching it from the exocoelomic mesenchyme (Dempsey, 1953).

The details of fine structure displayed by the cellular and syncytial elements of the placenta, both in the region of the subplacenta and in the walls of the decidual vessels, clearly illustrate the limits of the Grosser classification when the sites of transfer of materials of physiological importance between the maternal and foetal organisms are being considered. The electron microscope so far has scarcely been exploited in the elucidation in these complexities of placental structure.

SUMMARY

1. The fine structure of the guinea-pig subplacenta and the related parts of the chorio-allantoic placenta and basal decidua is described in the early and middle stages of gestation.

2. The cytotrophoblastic layer of the subplacenta is characterized by the dilated intercellular spaces, the finely granular cytoplasm, the numerous mitochondria, the large size of the nuclei relative to the cytoplasm, the multiple skein-like nucleoli, and the absence of glycogen and lipid. The subplacental syncytium is characterized by the relative paucity of mitochondria, the scattered areas of ergastoplasm, many electron dense droplets, large accumulations of glycogen and an absence of lipid. In addition, the syncytium is traversed by a complex and possibly anastomosing system of lacunae lined by microvilli and containing electron dense material which is strongly periodic acid-Schiff positive.

3. The highly modified walls of the maternal placental vessels are described at different levels. The lining of the maternal blood channels within the foetal placenta consists of overlapping sheets of chorionic epithelium. The lining of the vessels (endotrophoblast) as they pass through the subplacenta is syncytial. The walls of the vessels within the necrotic zone of the basal decidua consists of either (1) syncytial trophoblast alone, (2) maternal endothelium and syncytial trophoblast, (3) maternal endothelium and giant cytotrophoblastic cells, or (4) maternal endothelium alone.

4. The cells of the residual zone of the basal decidua are individually surrounded by a capsule of electron dense material which extends into the marginal bays and intracellular vacuoles of the cytoplasm. The relationship of these cells to the maternal vessels is described.

5. The endodermal cells of the parietal wall of the inverted yolk sac and the underlying Reichert's membrane and layer of chorionic giant cells are described.

6. The possible functional significance of the morphological specializations described in the subplacenta and in the vascular walls is discussed.

Grateful acknowledgement is made to Miss Sonia Michelson for technical assistance.

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EXPLANATION OF PLATES

The following abbreviations are used in the figures: B, bay of decidual cell; BM, basement membrane; CY, cytotrophoblast; DR, droplets; D, decidua; DC, decidual cell; DS, decidual space or cavity; DT, dark trophoblastic cell; DV, decidual vacuole; E, endoplasmic reticulum; EN, endothelium; ET, endotrophoblast; FM, foetal mesenchyme; FP, foot-process; FV, foetal vessel; G, glycogen; H, cell inclusion in chorionic giant cell; IC, intercellular space; L, syncytial lacuna; LT, light trophoblastic cell; M, mitochondrion; MV, maternal vessel; N, nucleus; PE, endoderm of parietal yolk sac; PM, plasma membrane; RM, Reichert's membrane; S, subplacental syncytium; ST, syncytial septum; SY, chorio-allantoic syncytium; TV, vacuole of chorionic giant cell; T, terminal bar. With the exception of Pl. 1, fig. 1, all figures are electron micrographs. All tissues were fixed in Dalton's fluid unless otherwise stated.

PLATE 1

Fig. 1. Subplacenta of the guinea-pig at the 25th day of gestation, drawn from a section viewed in the phase-contrast microscope. The cytotrophoblastic layer is multilaminar and rests on a thin basement membrane which separates it from the foetal mesenchyme. The intercellular spaces of this layer are dilated. The subplacental syncytium appears darker than the cytotrophoblast and contains vacuoles or lacunae and many small osmiophilic droplets. (Drawing by Mrs B. M. Velick.) ×500.

PLATE 2

Fig. 2. Subplacenta at about the 25th day from a typical area enclosed by black lines in Pl. 1, fig. 1. The cytotrophoblastic cells are characterized by their large nuclei, multiple skein-like nucleoli, dilated intercellular spaces, scattered mitochondria and granular cytoplasm. Terminal bars are found between adjacent cytotrophoblastic cells and between the plasma membranes of these cells and that of the syncytial trophoblast. Several large lacunae lined by microvilli are shown within the syncytium. × 5000.

PLATE 3

Fig. 3. Subplacenta at about the 35th day. The cytotrophoblastic layer is reduced to one cell in thickness and has disappeared in one area (Y), where the syncytium rests on the basement membrane by delicate cytoplasm or foot-processes. Similar processes of the syncytium also project into the marginal extracellular space (X) between this layer and the cytotrophoblastic layer. The true syncytial nature of the subplacental syncytium is evident. × 3000.

PLATE 4

Fig. 4. Details of the subplacenta syncytium at about the 37th day. The cytoplasm contains a few scattered mitochondria, islands of ergastoplasm, numerous electron dense droplets and large accumulations of glycogen. The larger lacunae are lined by microvilli and contain material of medium electron density. Other lacunae have smooth walls and are filled with more electron dense material. × 5000.

PLATE 5

Fig. 5. Details of the junction between the cytotrophoblast of the subplacenta (to the right) and the syncytium (to the left). 37th day. The marginal extracellular space between the two layers (X) is narrow, contains small processes of other cells and is continuous with the dilated intercellular spaces between the cytotrophoblastic cells. These spaces are partially lined by microvilli derived from the limiting plasma membrane of the syncytium. The cytoplasm of the cytotrophoblastic cells is granular with scattered vesicles. The mitochondria of these cells are larger than those of the syncytium. The syncytial cytoplasm contains scattered mitochondria, masses of glycogen and droplets of varied electron density. × 11,000.

PLATE 6

Fig. 6. Subplacenta at about the 54th day. A group of cytotrophoblastic cells occupy the upper part of the figure. Elsewhere the syncytium rests directly on the basement membrane by small foot-processes. Terminal bars are found where the plasma membranes of the cytotrophoblastic cells and of the syncytium come into contact. The basement membrane of the trophoblast is cut tangentially and is fibrillar. × 4000.

Fig. 7. Details of the subplacenta syncytial lacunae (54 days). The lacunae are filled with dense material in which are embedded the lining microvilli. The lacunae appear to communicate to some extent and may form a confluent system. Palade's fluid. × 6000.

PLATE 7

Fig. 8. The basement membrane of the trophoblast is cut tangentially and appears amorphous. In it are embedded the foot-processes of the subplacental syncytium which enclose a labyrinthine extracellular space. Palade's fluid. × 10,000.

The following figures (9 to 20) illustrate the character of the epithelium lining the maternal blood channels, beginning within the chorio-allantoic placenta and proceeding toward the myometrium.

Fig. 9. Area of 'fine syncytium' from the chorio-allantoic placenta at about the 35th day. The lining of the maternal blood channels is made up of the imbricated trophoblastic cells and is not syncytial. Irregularly dilated spaces within the cytoplasm (X) indicate intercellular spaces. Small electron dense bodies are embedded between the microvilli at the luminal edge of the trophoblast. × 2500.

Fig. 10. Area of 'coarse syncytium' (no foetal vessels) within the chorio-allantoic placenta at the 35th day. The lining of the maternal blood spaces may consist of syncytial trophoblast or of imbricated sheets of cytotrophoblastic cells. The linear dense areas within the cytoplasm (at X) may represent the plasma membranes of overlapping cells. The electron dense bodies between the microvilli are angular in profile, suggesting a crystalline structure. × 10,000.

PLATE 8

Fig. 11. Wall of a large maternal placental vessel passing through the subplacenta (about 35 days). The vessel is lined by a thick layer of syncytial trophoblast (endotrophoblast). The syncytial cytoplasm appears vacuolated due to the presence of swollen mitochondria and dilated vesicles and tubules of the endoplasmic reticulum. Many of the dilated spaces of the endoplasmic reticulum contain amorphous material (at X). A group of cytotrophoblastic cells is shown in relation to the basement membrane. × 4000.

Fig. 12. Dilated syncytial lacunae within the subplacenta in immediate relation to a large maternal vessel (about 35 days). The syncytial trophoblast separating the lacunae are extremely attenuated. The lacunae contain a flocculum of precipitated material and masses of dense material (X). × 4000.

PLATE 9

Fig. 13. Wall of a large maternal vessel (first type) within the necrotic zone of the basal decidua close to the subplacenta (45 days). The endotrophoblastic lining of the vessel is syncytial. The cytoplasm contains swollen mitochondria, occasional tubular and vesicular elements of the endoplasmic reticulum, and dense accumulations of granules. × 10,000.

Fig. 14. Portion of wall of large maternal vessel (second type) within the necrotic zone of the basal decidua (45 days). The vessel is lined by maternal endothelium external to which is syncytial trophoblast (endotrophoblast). A thin basement membrane lies between the endothelium and the endotrophoblast. The marginal cytoplasm of the latter is inserted into the endothelial basement membrane by foot-processes enclosing an extracellular space. This space communicates at some points (X) with the dilated cisterns and tubules of the endoplasmic reticulum. × 10,000.

PLATE 10

Fig. 15. Portion of wall of a maternal vessel (second type) within the necrotic zone of the basal decidua (45 days). The endotrophoblastic cytoplasm is less vacuolated than in Pl. 9, fig. 14. The foot-processes of the endotrophoblast related to the endothelial basement membrane are shown. × 14,000.

Fig. 16. Wall of maternal placental vessel (second type) within the necrotic zone of the basal decidua (45 days). The wall consists of maternal endothelium and of endotrophoblast which is syncytial. The dilated tubules and cisterns of the endoplasmic reticulum are very conspicuous in the endotrophoblast. At the lower edge of the figure the endotrophoblast fades out, leaving only the maternal endothelium, and the two basement membranes (that of the endothelium, that of the endotrophoblast) become confluent. × 2500.

Fig. 17. Wall of maternal vessel (fourth type) within the basal decidua consisting solely of maternal endothelium. The plasma membranes of adjoining cells interlock with no visible terminal bars. Lipid inclusions are found within the endothelial cytoplasm. × 8000.

Fig. 18. Wall of maternal vessel within the residual zone (normal though modified) zone of the basal decidua, close to the myometrium. An extensive extracellular space intervenes between the basement membrane of the maternal endothelium and the plasma membranes of the decidual cells. The latter are inflected into the decidual cytoplasm in the form of bays and are filled with amorphous material. Wisps of collagen and similar accumulations of amorphous material occupy the sub-endothelial space. × 8000.

PLATE 11

Fig. 19. Wall of maternal placental vessel (third type) within the necrotic zone of the basal decidua (46 days). The maternal endothelium is thick and finely granular. One endothelial cell contains swollen mitochondria and large vacuoles. External to the endothelium is the endotrophoblast made up of several layers of giant cytotrophoblastic cells. These are of two types: light

and dark, based on the electron density of the cytoplasm and the development of the endoplasmic reticulum. Foot-processes of the endotrophoblastic cells are implanted on to the endothelial basement membrane (at F). The labyrinthine extracellular space between the processes is continuous with the dilated intercellular spaces between the giant cytotrophoblastic cells. Vacuoles are present within the marginal cytoplasm of the endothelium and the light cytotrophoblastic cells. × 10,000.

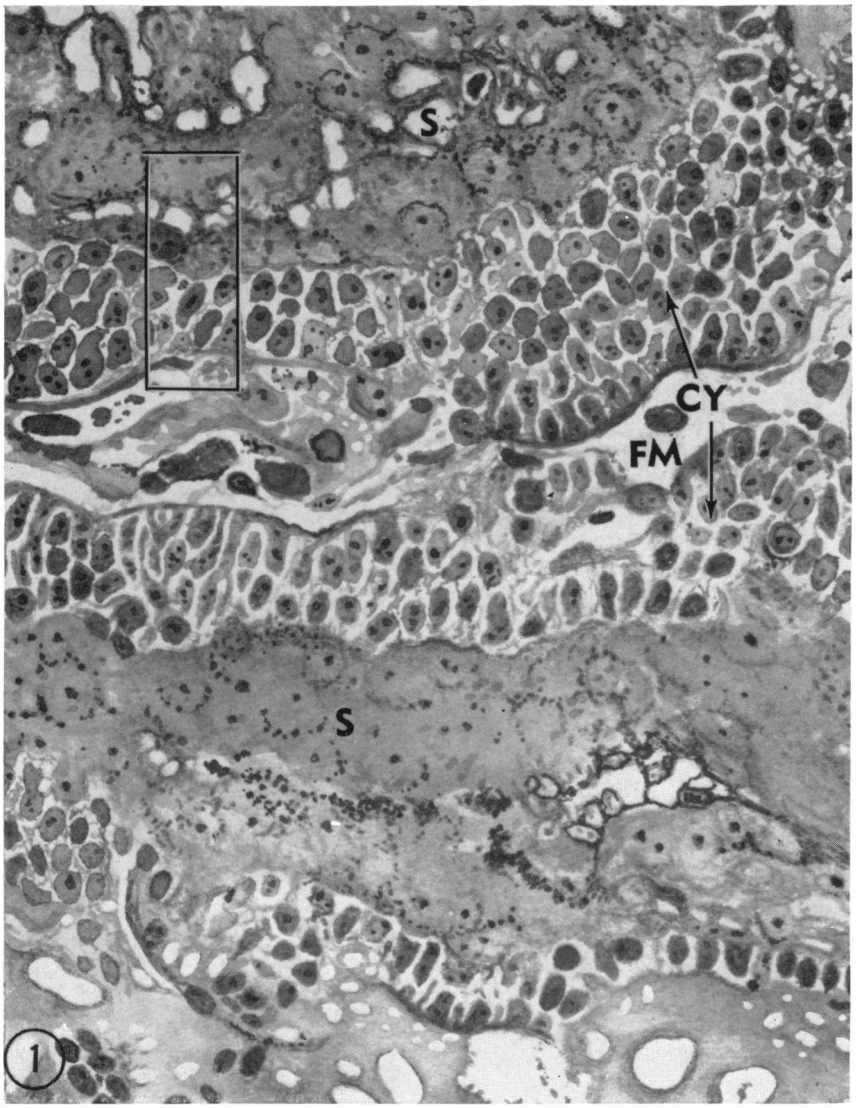
PLATE 12

Fig. 20. Wall of maternal vessel within the residual zone of the basal decidua (45 days). The lining endotrophoblast is syncytial and is very electron dense, perhaps the result of degenerative changes. It is related externally to normal decidual cells, a wide extracellular space intervening (see Pl. 10, fig. 18). × 2500.

Fig. 21. Decidual cells within the residual zone of the basal decidua (36 days). The plasma membranes are sinuous and interlock in a complex manner. They are inflected into the cytoplasm as recesses and bays which contain material of marked electron density. These bays in some cases may be traced into continuity with large intra-cytoplasmic vacuoles lined by microvilli and containing granular material of low electron density. The decidual cytoplasm contains a few mitochondria, scattered electron dense inclusions (pigment?) and glycogen. × 2500.

PLATE 13

Fig. 22. Section through the layer of endodermal cells comprising the parietal wall of the inverted yolk sac and through the underlying layer of chorionic giant cells. The surface of the endoderm facing the decidual cavity is thrown up into long microvilli. The intercellular spaces of this layer are dilated and contain amorphous material continuous with Reichert's membrane. The giant cytotrophoblastic cells contain perinuclear aggregations of endoplasmic reticulum, homogeneous bodies of varying electron density enclosed by definite membranes, scattered mitochondria, and large vacuoles containing small electron dense inclusions. × 10,000.



DAVIES, DEMPSEY AND AMOROSO—THE SUBPLACENTA OF THE GUINEA PIG

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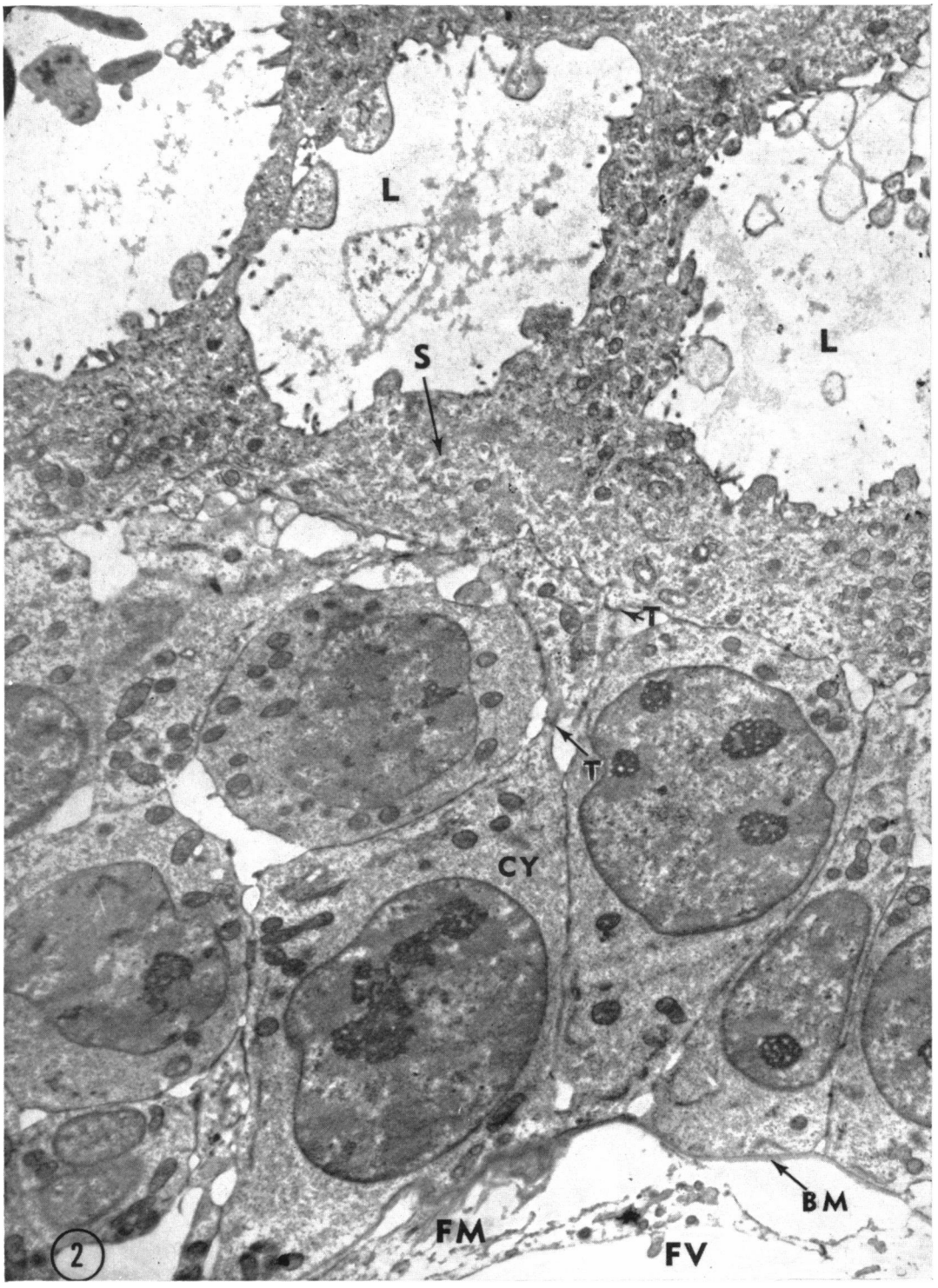

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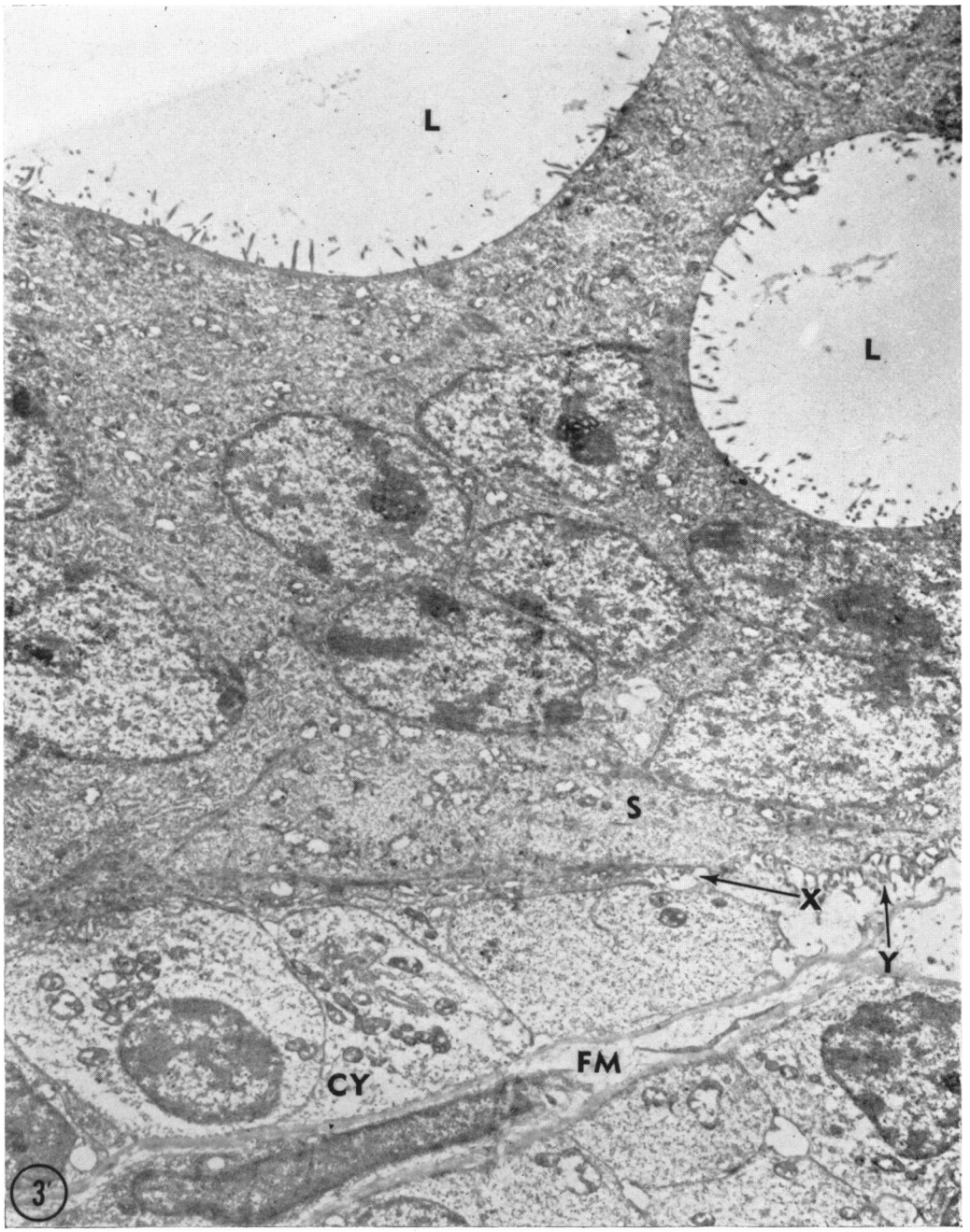

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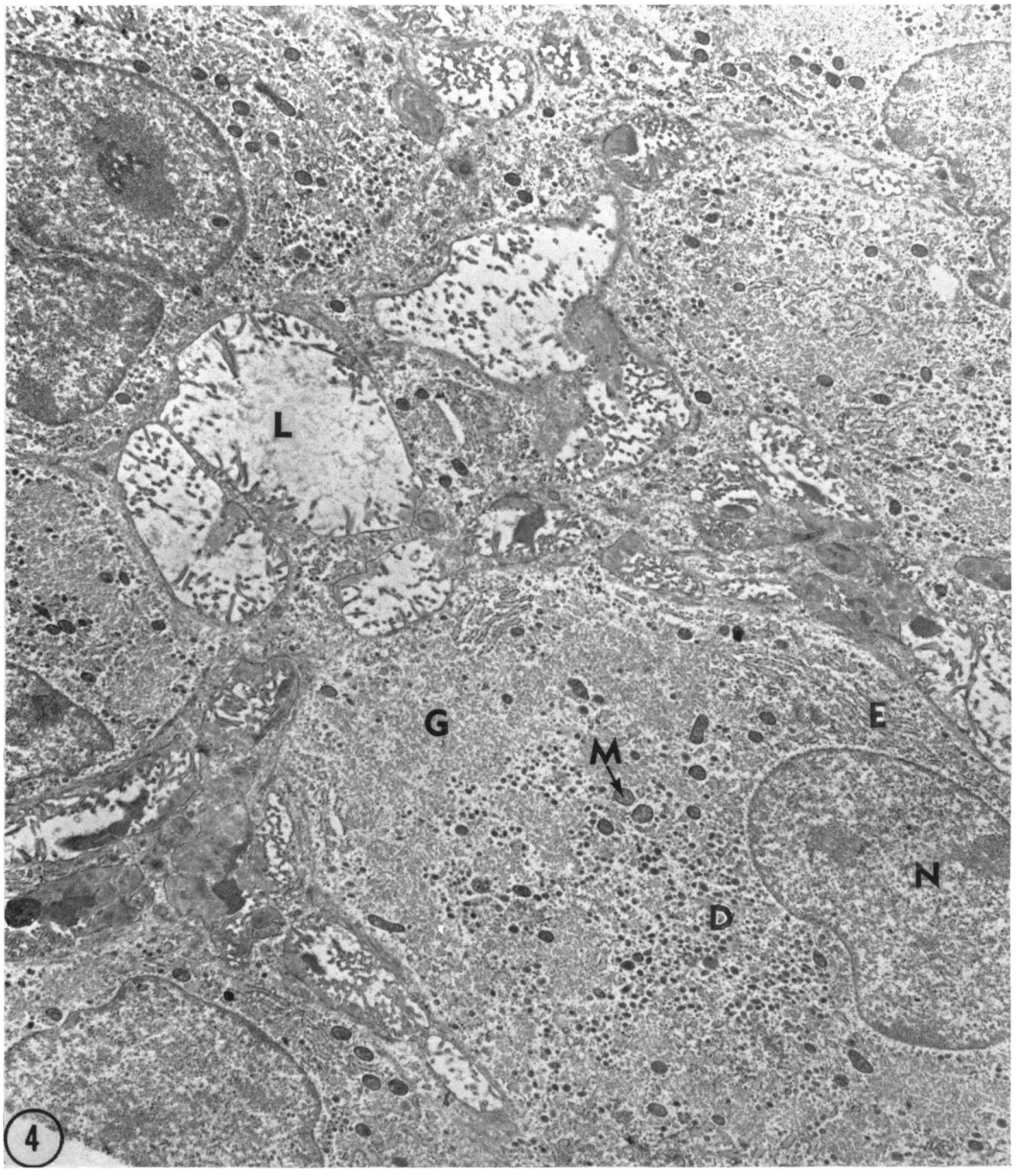

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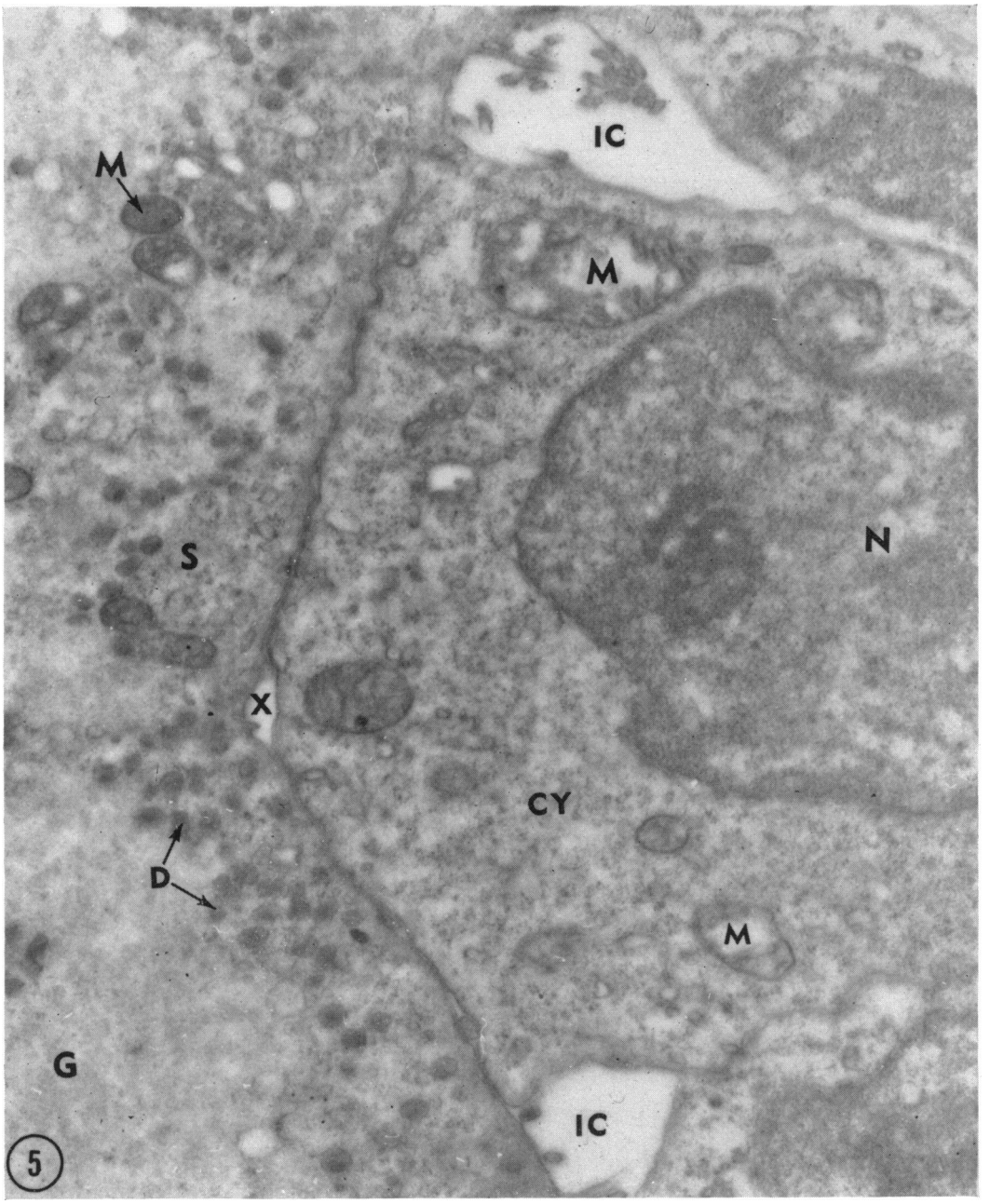

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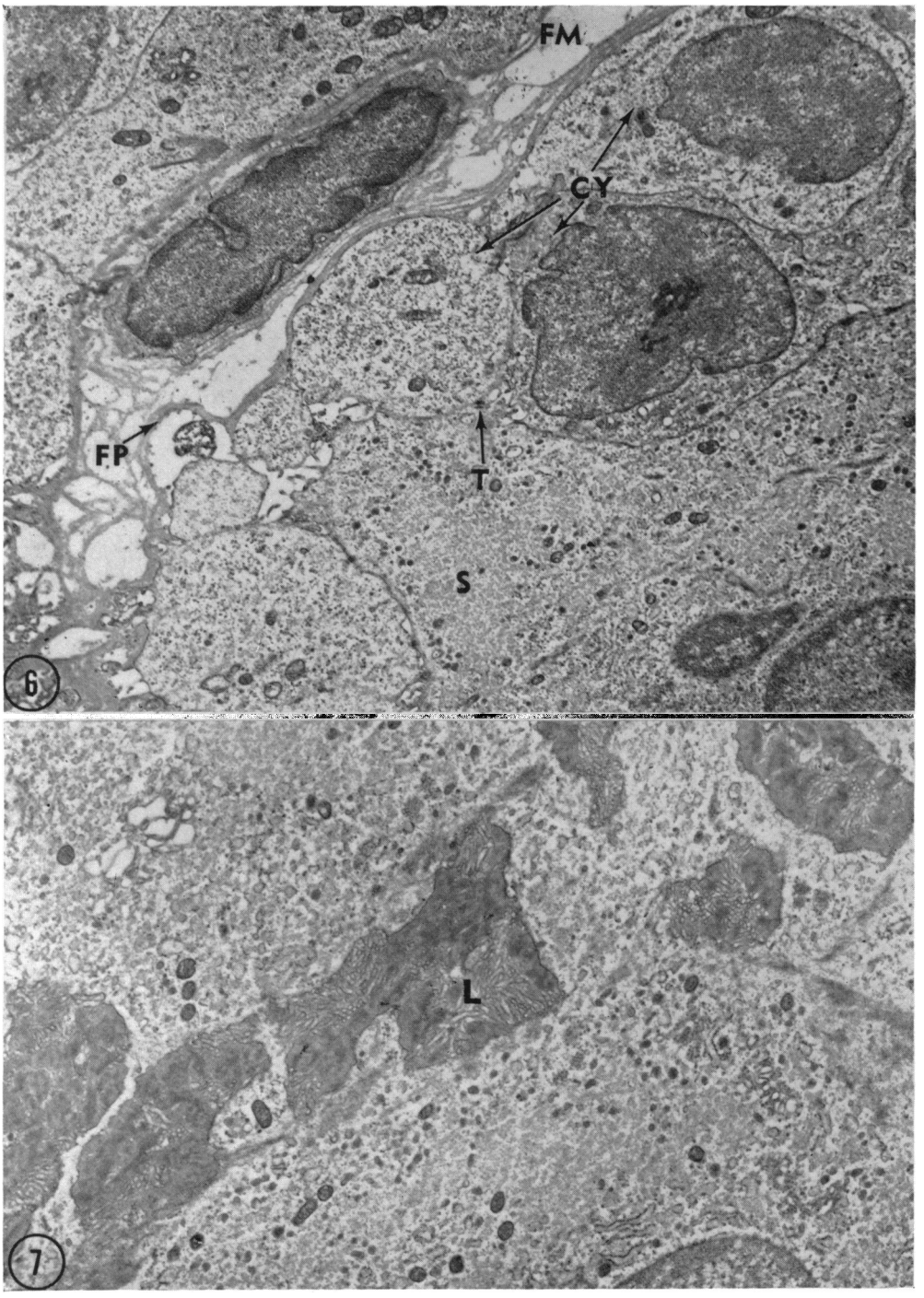

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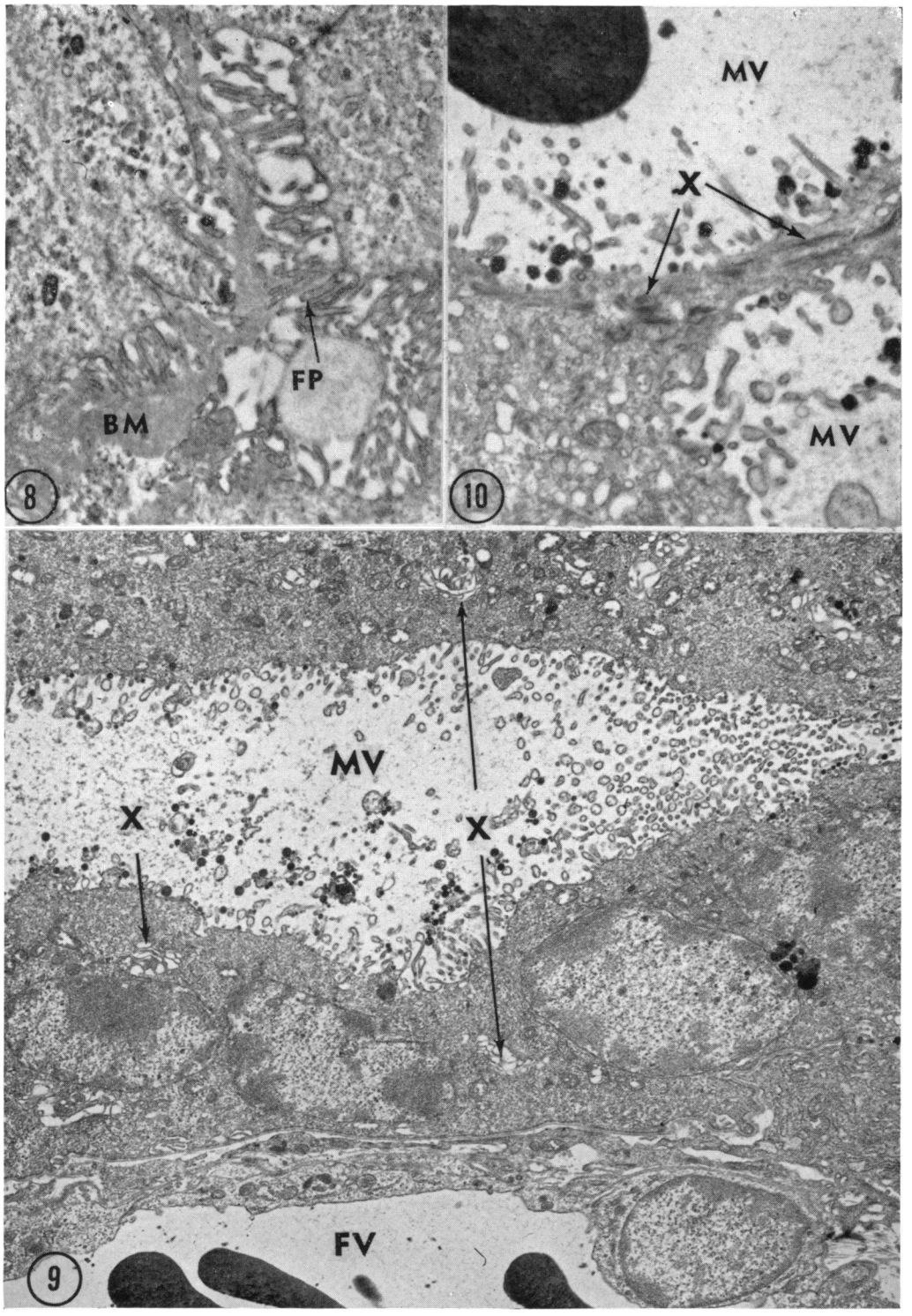

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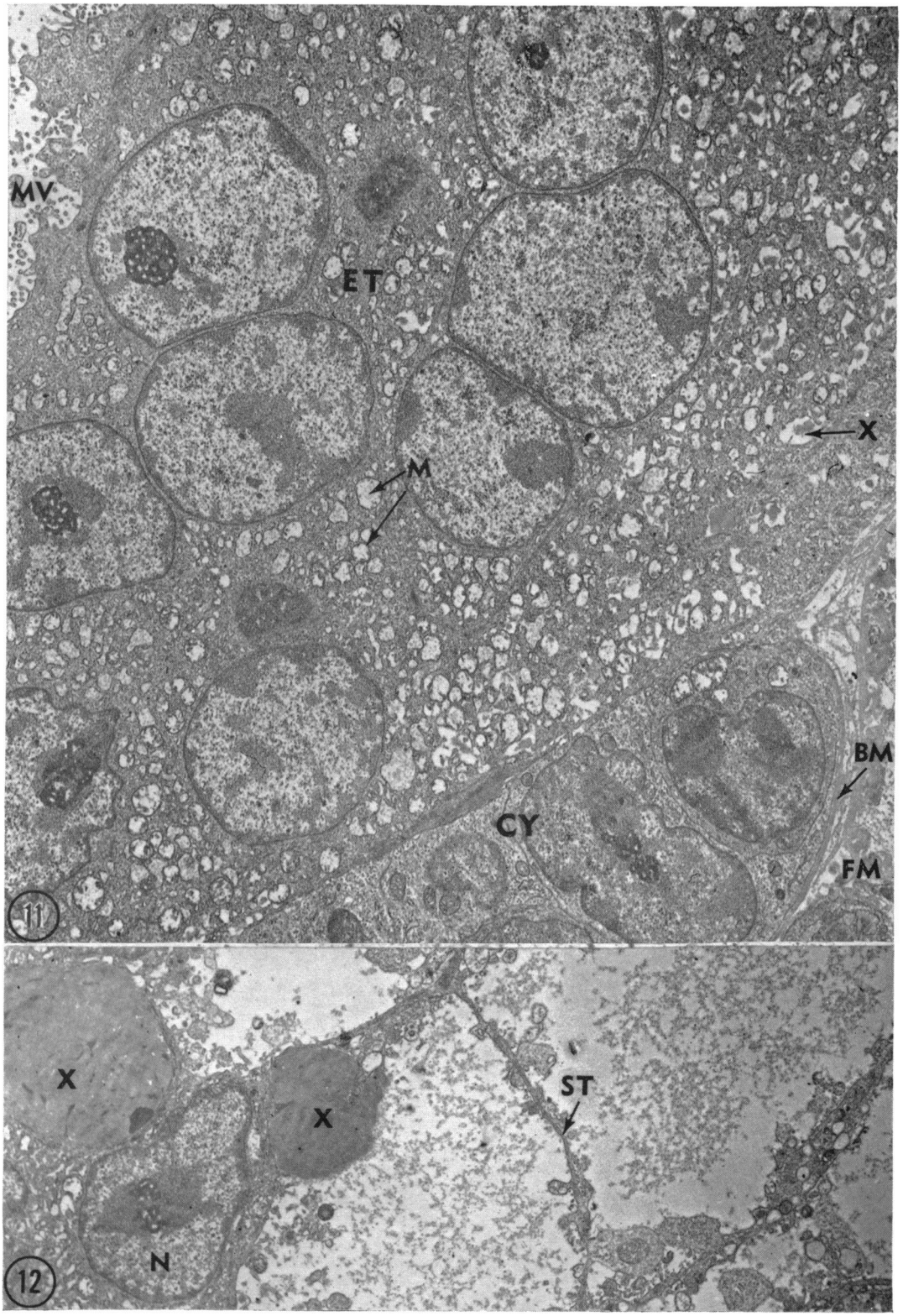
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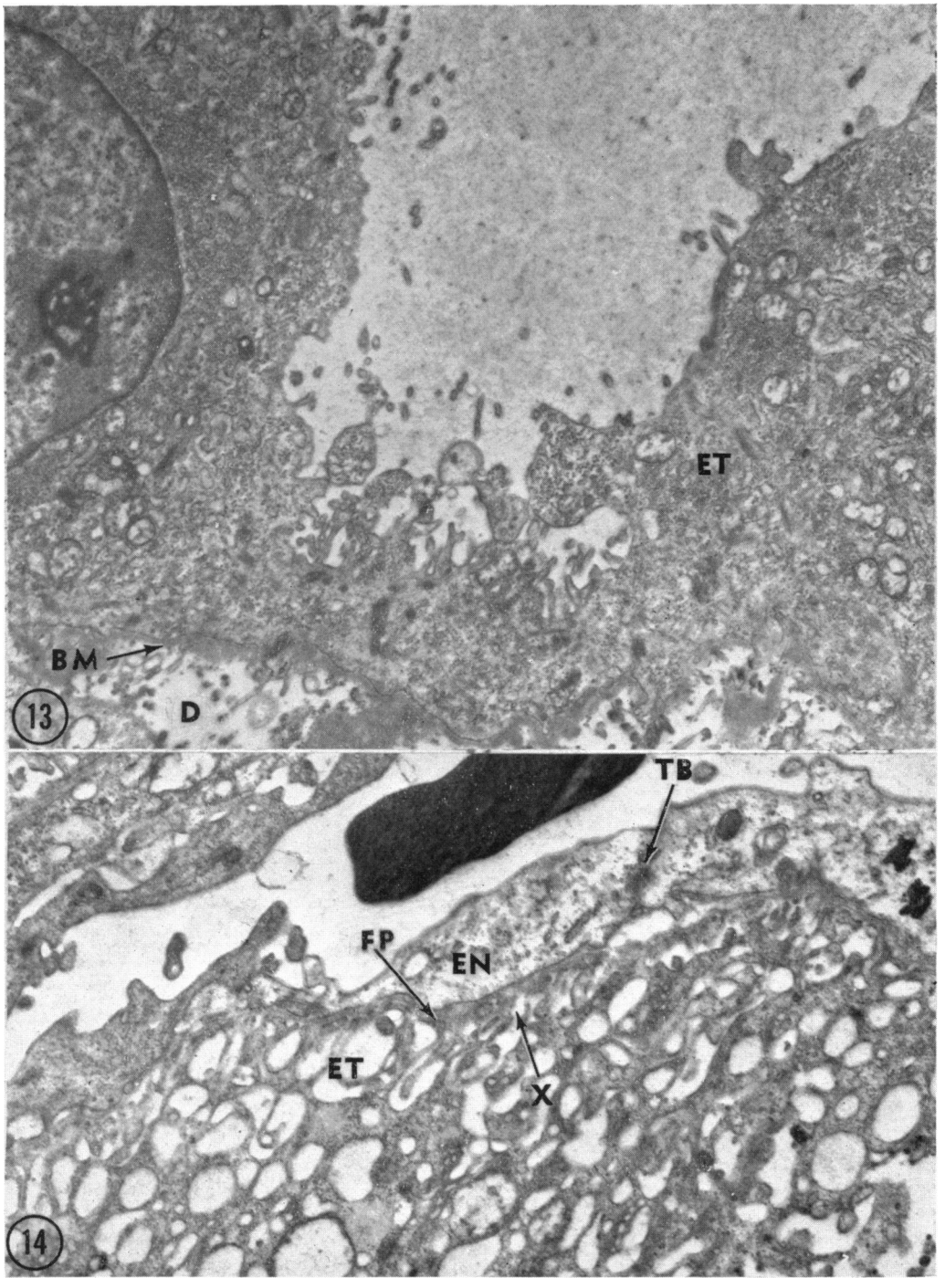

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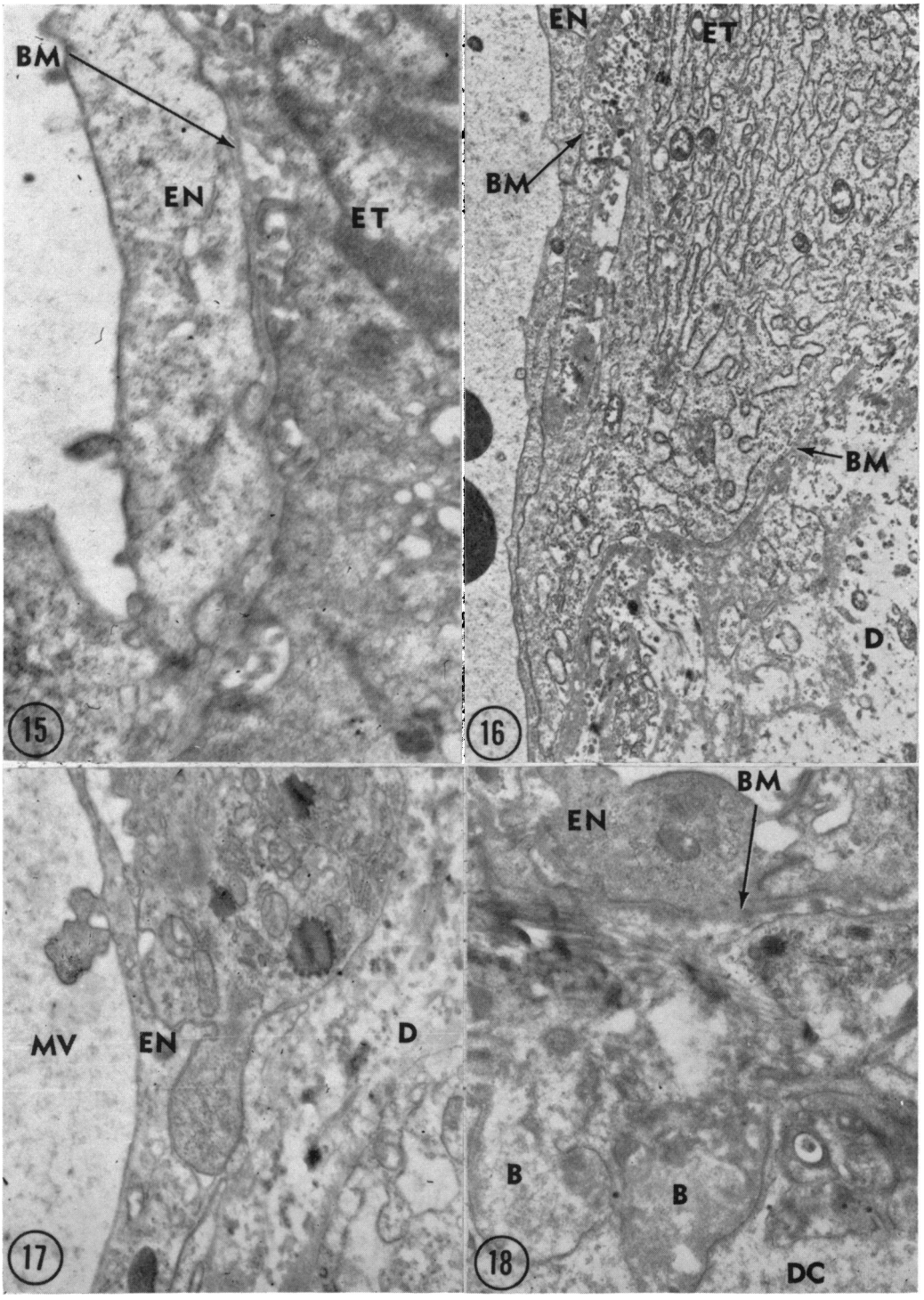

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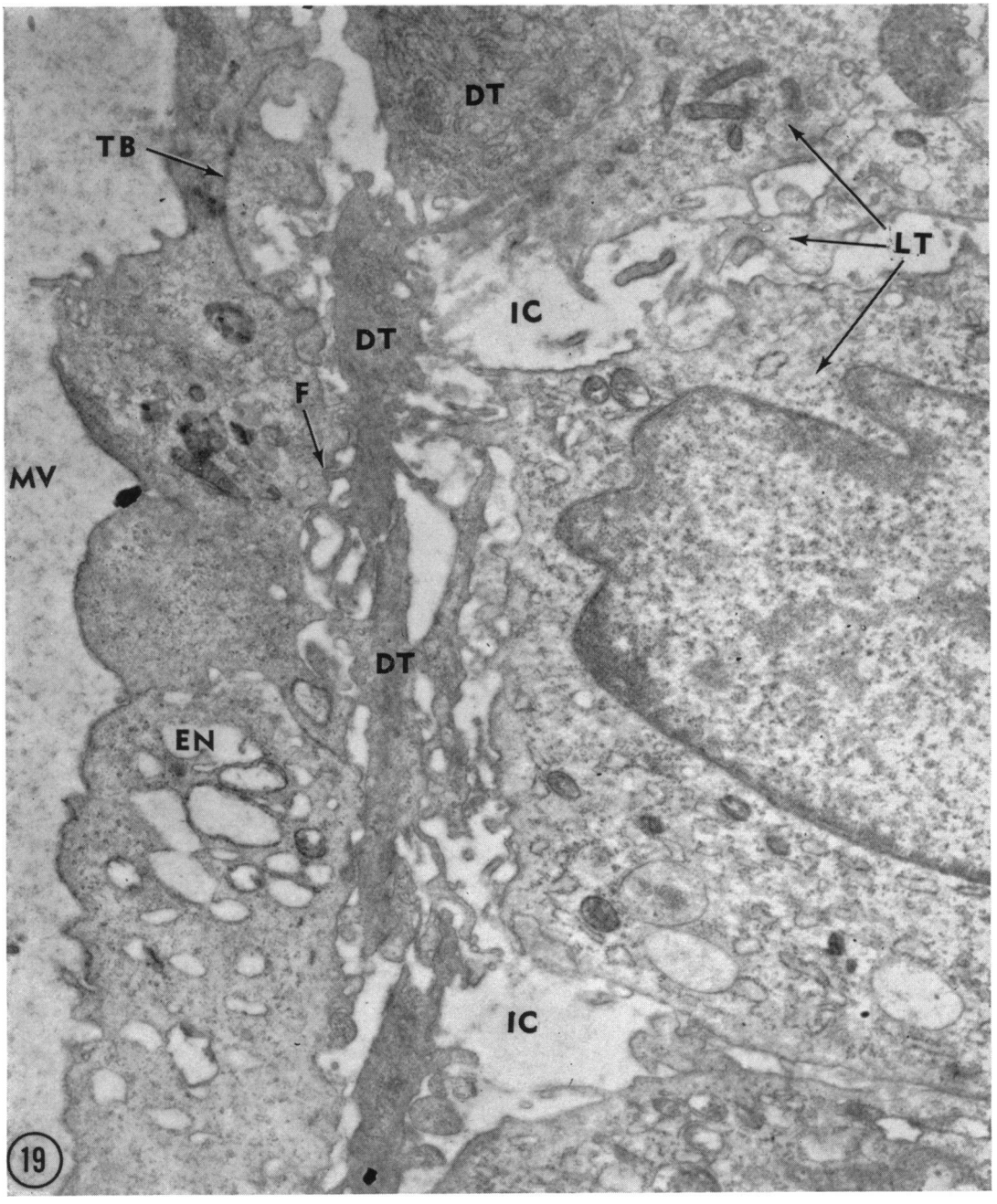

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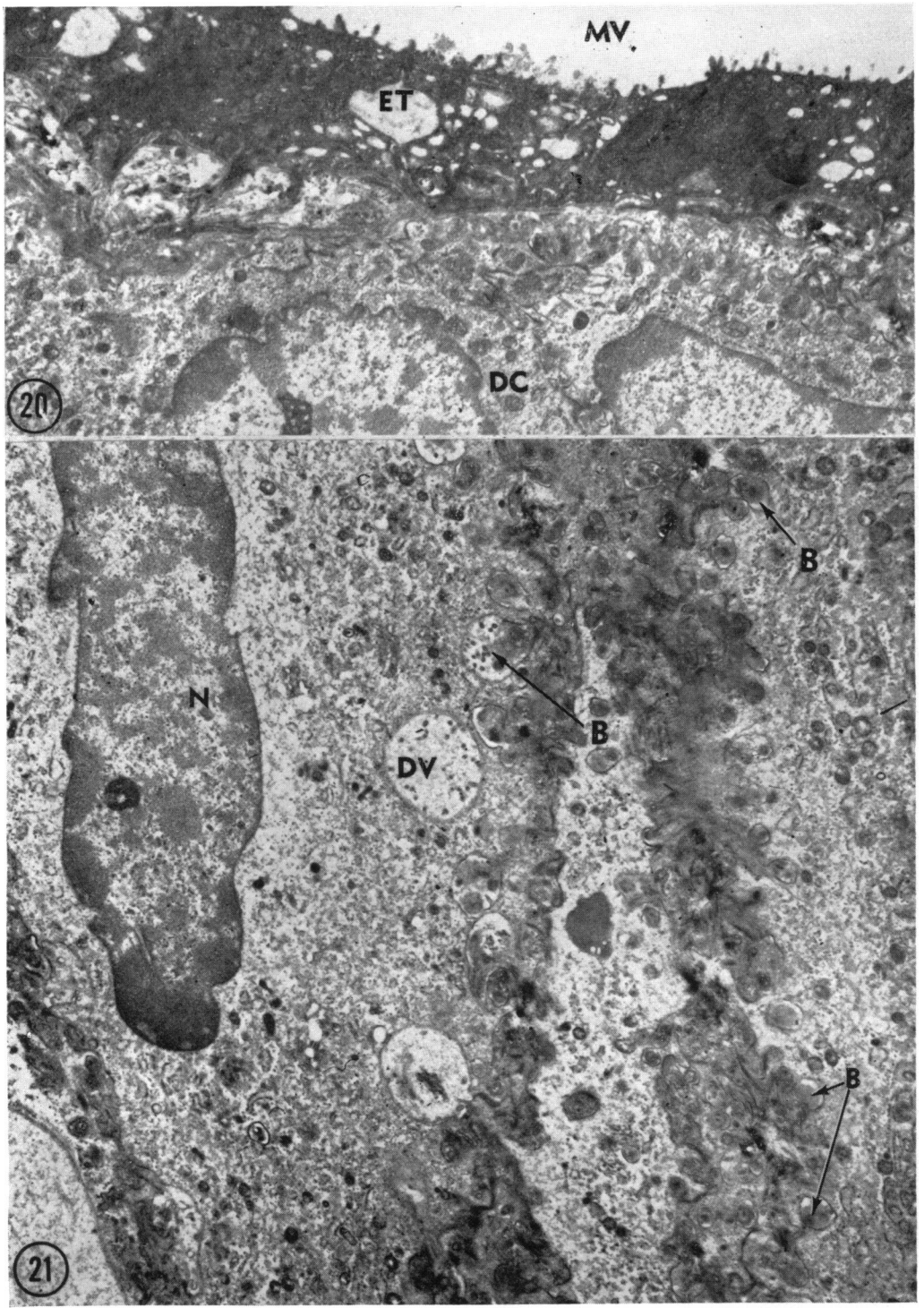

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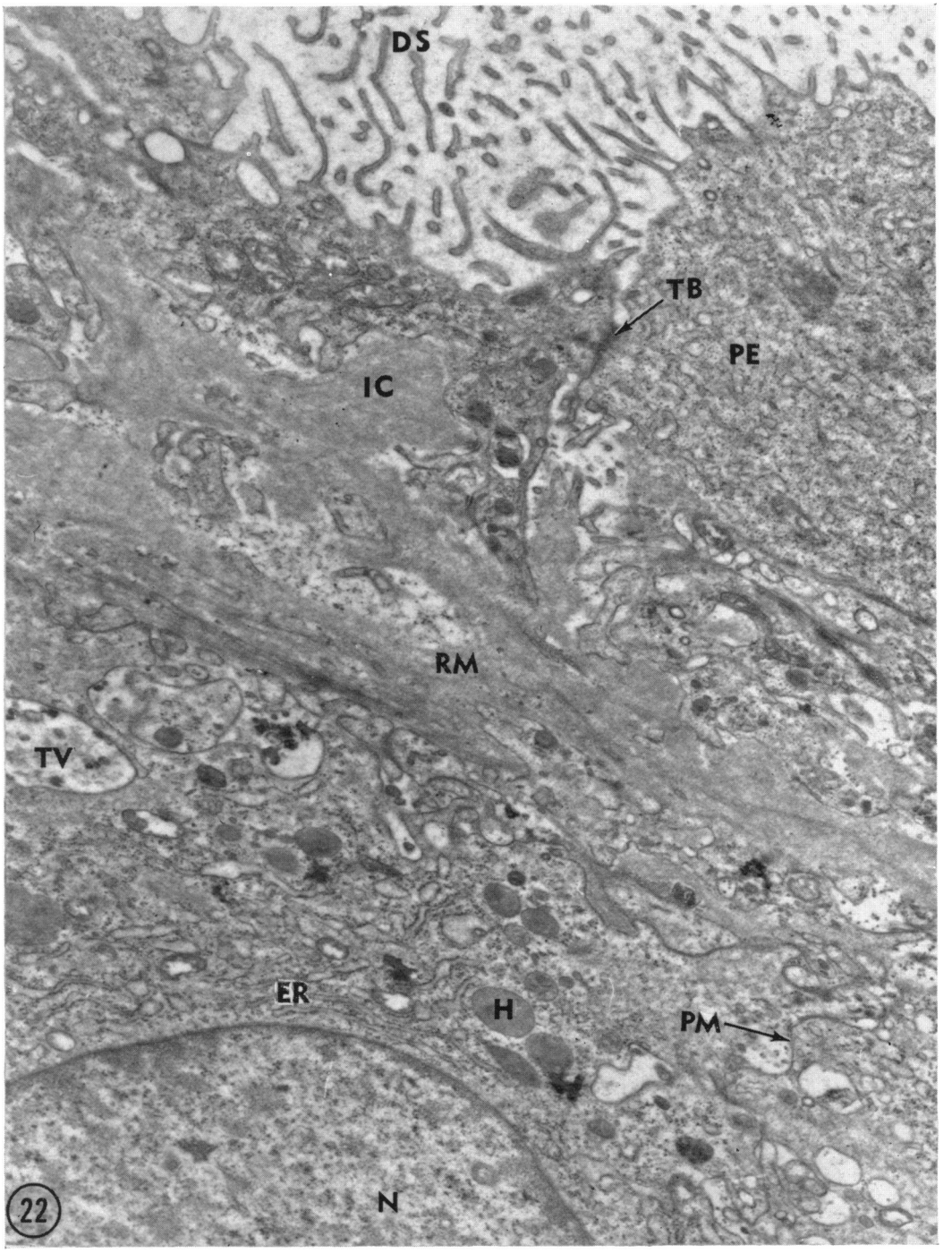

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