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A morphological study of granulated metrial gland cells and trophoblast cells in the labyrinthine placenta of the mouse

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INTRODUCTION

Granulated metrial gland (GMG) cells are usually associated with the decidua basalis and metrial gland of the pregnant rodent uterus (Dickson & Bulmer, 1961; Stewart & Peel, 1980), where they differentiate *in situ* from cells which have been shown to originate in the bone marrow (Peel, Stewart & Bulmer, 1983). Proliferation of GMG cells within the decidua basalis, and later in the metrial gland, is thought to contribute to the increase in numbers of these cells (Stewart & Peel, 1980). However, neither the function nor the sites of functional activity of GMG cells have been established. Some of the GMG cells in the decidua basalis and metrial gland migrate into the lumina of blood vessels and pass to the maternal blood spaces of the fetal placenta, including the labyrinth (Stewart & Peel, 1978). GMG cells are found in greatest numbers during the growth period of the labyrinth and more are found in outbred pregnancies than in corresponding inbred pregnancies (Jbara & Stewart, 1982). A preliminary study has identified changes in the morphology of some labyrinthine trophoblast lying next to GMG cells (Stewart & Jbara, 1980).

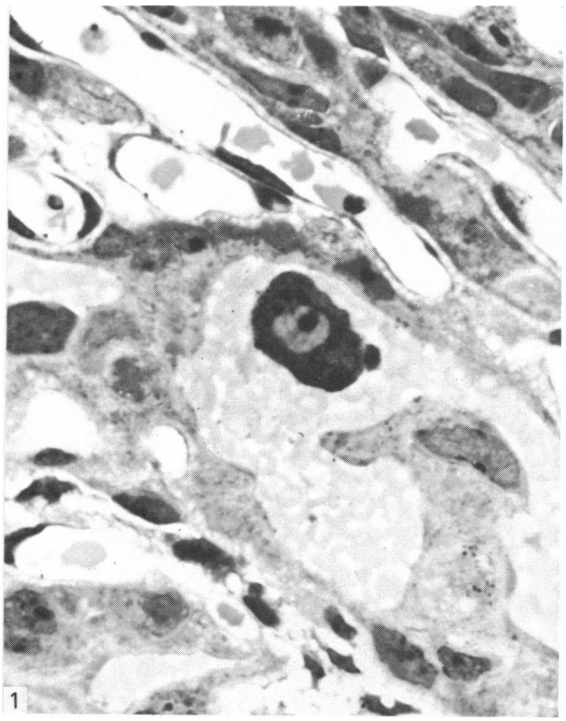
In the present study, the possibility that the labyrinthine placenta is a site of functional importance to GMG cells has been explored using both light and electron microscopy. In particular, morphological changes in some GMG cells and in some trophoblast cells have been examined and an attempt made to assess the spatio-temporal relationships of these cells.

MATERIALS AND METHODS

Nulliparous mice were mated overnight (Porton ♀ × Porton ♂; LAC129 ♀ × Obese ♂; LAC129 ♀ × LAC129 ♂) and the morning on which a vaginal plug was detected was designated Day 0 of pregnancy. Mice were killed by cervical dislocation at Day 10 (9 am), Day 10½ (9 pm) and on Days 11, 12, 13, 14 or 17 of pregnancy. Implantation sites were removed and fixed for 8–24 hours in a mixture of 2% glutaraldehyde and 4% formaldehyde in 0·1 M phosphate buffer, pH 7·2–7·4 (modified from Karnovsky, 1965).

Light microscopy

Transverse slices of implantation sites were dehydrated in ethanol and embedded in glycol methacrylate (Rudell, 1967). Transverse sections, 1 μm thick, of implantation sites were reacted with the periodic acid–Schiff technique, with or without previous diastase digestion, and counterstained with haematoxylin.



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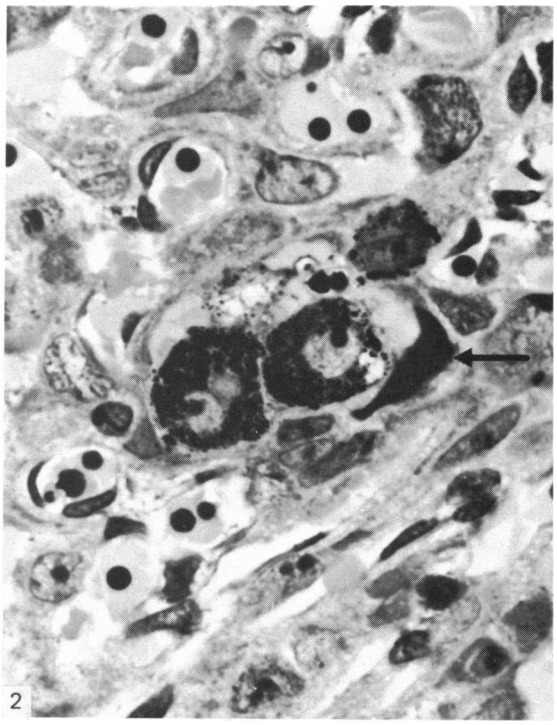

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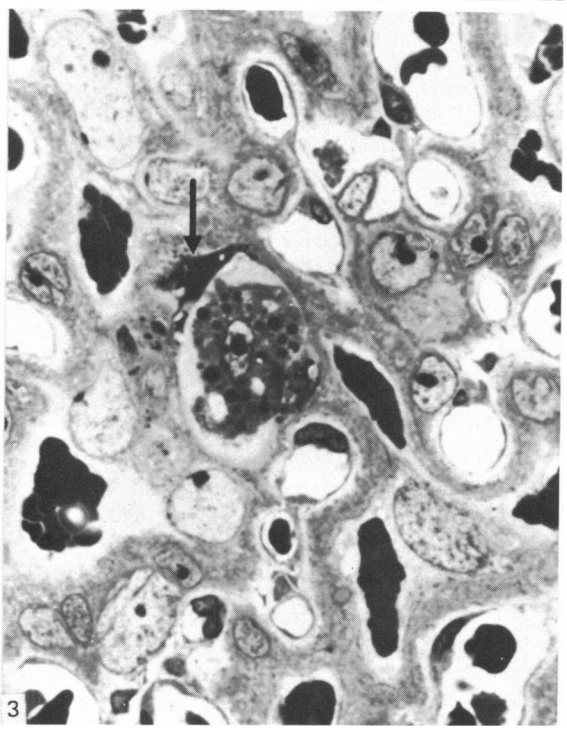

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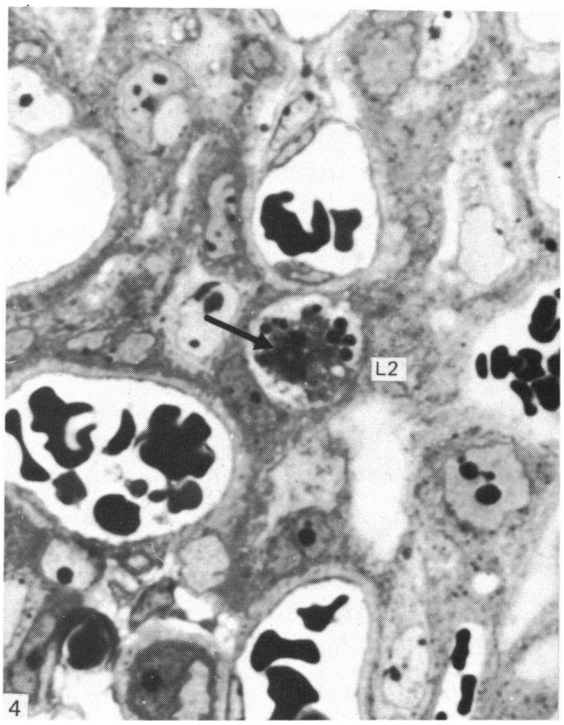

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Table 1. *The spatiotemporal relationship of GMG cells and trophoblast in the labyrinthine placenta*

Day of pregnancy	Number of animals	A	B	C	D
10	6	21	2	0	0
10½	3	21	8	3	1
12	1	16	8	1	3
14	3	56	28	13	7
17	3	22	8	11	10

(A) Healthy GMG cell not associated with densely stained trophoblast.
(B) Healthy GMG cell associated with densely stained trophoblast.
(C) Degenerate GMG cell associated with densely stained trophoblast.
(D) Degenerate GMG cell not associated with densely stained trophoblast.

Following an initial analysis of the sectioned material, ten sections of implantation sites from Porton mice killed at Day 10, Day 10½, Day 12, Day 14 and Day 17 of pregnancy were prepared with no more than two sections, each spaced at least 50 μm apart, prepared from any one block of tissue. The number of GMG cell profiles which showed both a nucleus and at least one of the typical glycoprotein granules was determined and an assessment made of the condition of each GMG cell and of the trophoblast cells lying next to it. Each GMG cell was then assigned to one of the following groups.

(*a*) Healthy GMG cell not associated with densely stained trophoblast.
(*b*) Healthy GMG cell associated with densely stained trophoblast.
(*c*) Degenerate GMG cell associated with densely stained trophoblast.
(*d*) Degenerate GMG cell not associated with densely stained trophoblast.

Serial sections, 1 μm thick, were prepared of part of an implantation site from mice killed on Days 12 or 14 of pregnancy (one site from each Porton × Porton and LAC129 × Obese pregnancy at each stage). Every fifth section was mounted and examined to identify any densely stained trophoblast cells in the labyrinth which did not appear to be associated with a GMG cell. The position of such cells was noted and neighbouring sections were examined to determine whether or not any GMG cells were associated with them.

Electron microscopy

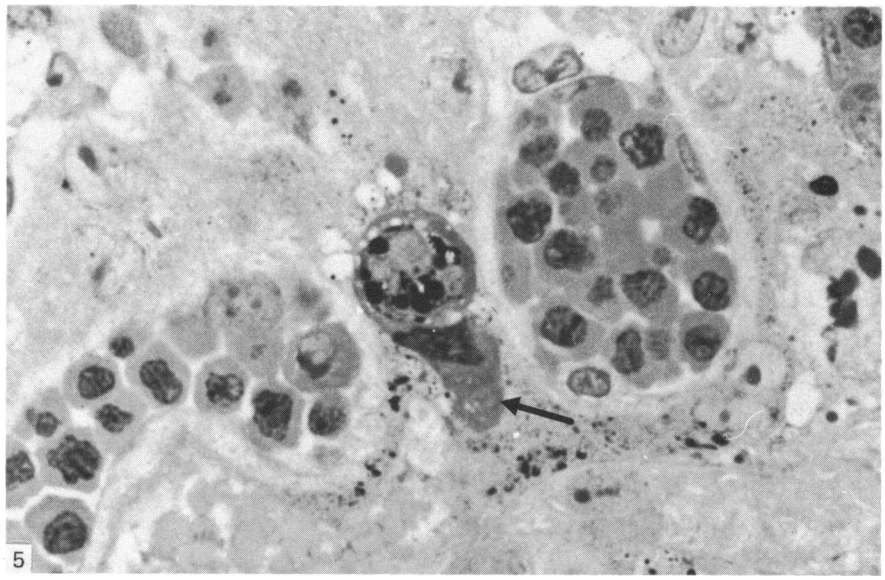
Pieces of labyrinthine placenta from animals killed on Days 12, 13 or 14 of pregnancy were postfixed in 1% osmium tetroxide, block stained in 2% uranyl

Fig. 1. An apparently healthy GMG cell, containing glycoprotein granules and glycogen, which appears to be lying freely within the lumen of a maternal blood space in the labyrinth. Periodic acid–Schiff and haematoxylin. Day 14 of pregnancy. × 750.

Fig. 2. Two apparently healthy GMG cells, containing glycoprotein granules and glycogen, are shown in a maternal blood space in the labyrinth. A densely stained trophoblast cell is seen (arrowed). Periodic acid–Schiff and haematoxylin. Day 14 of pregnancy. × 750

Fig. 3. A degenerate GMG cell with densely stained cytoplasm is shown lying next to a densely stained trophoblast cell (arrow). Toluidine blue. Day 14 of pregnancy. × 750.

Fig. 4. A degenerate GMG cell. The nucleus (arrow) is irregular in shape and densely stained. L2, layer 2 trophoblast. Toluidine blue. Day 14 of pregnancy. × 750.



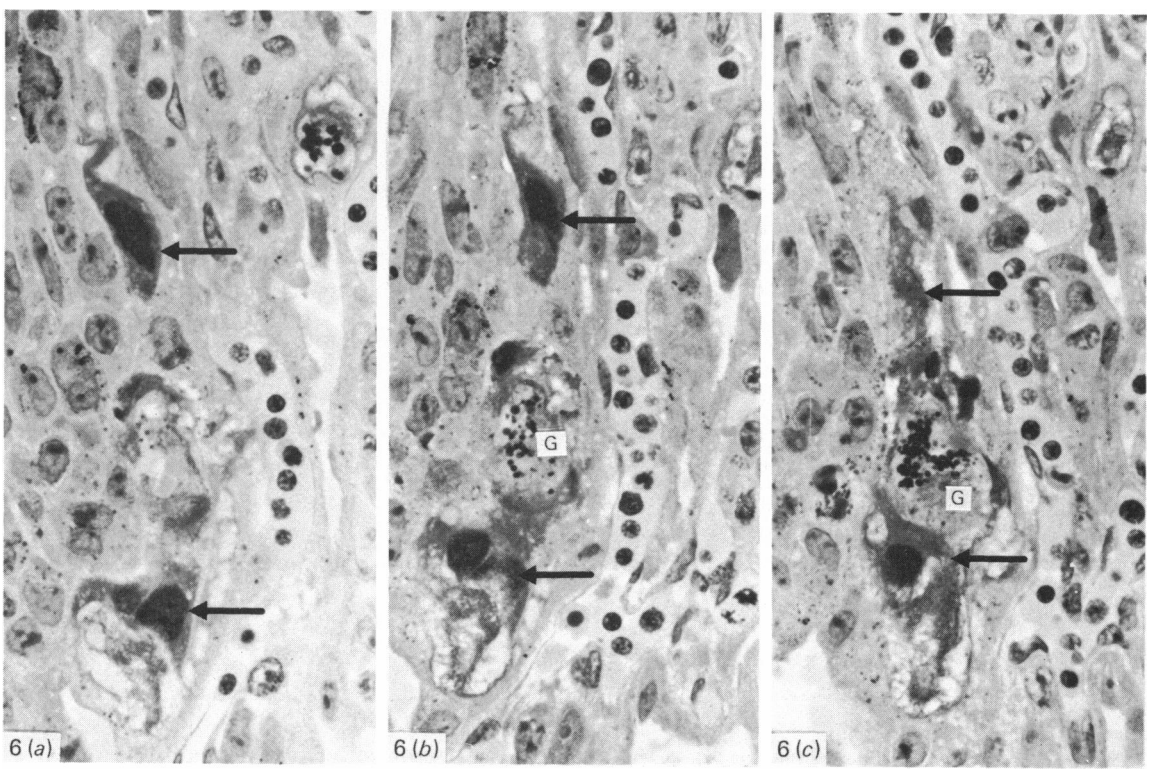


Fig. 5. A densely stained degenerate trophoblast cell (arrow) with a GMG cell in the developing labyrinth at Day 10 of pregnancy. Periodic acid–Schiff and haematoxylin. × 800.

Fig. 6 (*a–c*). Densely stained trophoblast cells (arrows) in the labyrinth. A GMG cell (G) is not present, adjacent to the densely stained trophoblast cells, in Fig. 6*a* but is present in neighbouring sections (Fig. 6*b*, +5 μm; Fig. 6*c*, +10 μm). Periodic acid–Schiff and haematoxylin. Day 12 of pregnancy. × 520.

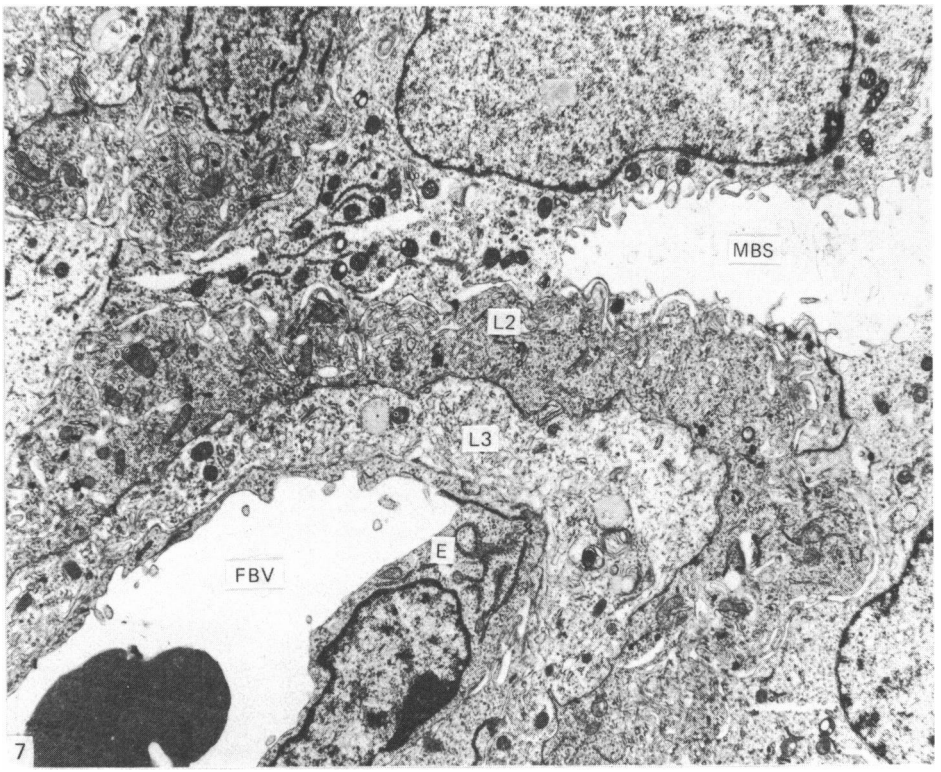


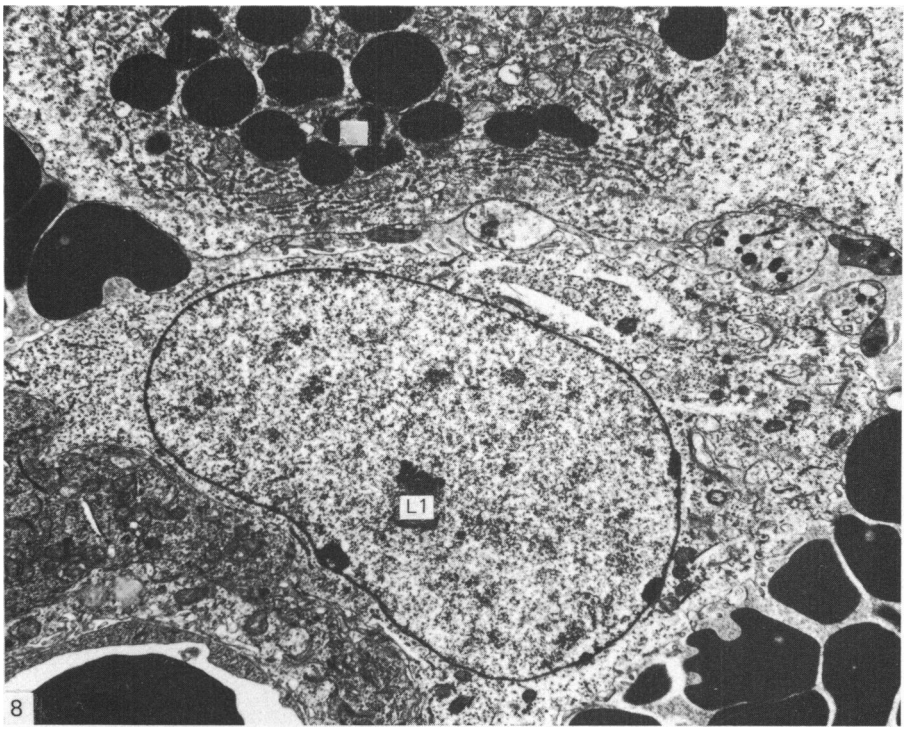
Fig. 7. The cellular barrier in the labyrinth between the lumen of a maternal blood space (MBS) and the lumen of a fetal blood vessel (FBV). The palely stained layer 1 trophoblast (L1) lines the maternal blood space. L2, layer 2 trophoblast; L3, layer 3 trophoblast; E, endothelium. ×5100.

acetate, dehydrated in acetone and embedded in Araldite. Sections, 1 μm thick, were stained with 1 % toluidine blue in 1 % borax for examination with the light microscope. Silver-gold sections were prepared of areas of labyrinth, with or without GMG cells, stained with uranyl acetate and lead citrate and examined with the electron microscope.

RESULTS

GMG cells were found in the labyrinthine placenta at each of the stages of pregnancy examined. Some GMG cells were surrounded by blood cells in the lumina of maternal blood spaces (Fig. 1) but others were closely apposed to trophoblast cells lining the lumen. Often the individual cellular layers of the maternofetal barrier were not clearly discernible and the precise cellular associations of the GMG cells could not be determined. The majority of the GMG cells in the labyrinth appeared healthy (Table 1), with one or two rounded nuclei often containing a prominent nucleolus. The pale staining cytoplasm contained glycogen and numerous glycoprotein granules (Figs. 1, 2). However, some GMG cells appeared to be degenerate; the nuclei were densely stained and irregular in shape and the cytoplasm was more densely stained and vacuolated (Figs. 3, 4). The proportion of GMG cells which appeared degenerate was higher with increased gestational age (Table 1).

The vast majority of layer 1 labyrinthine trophoblast cells which were identified



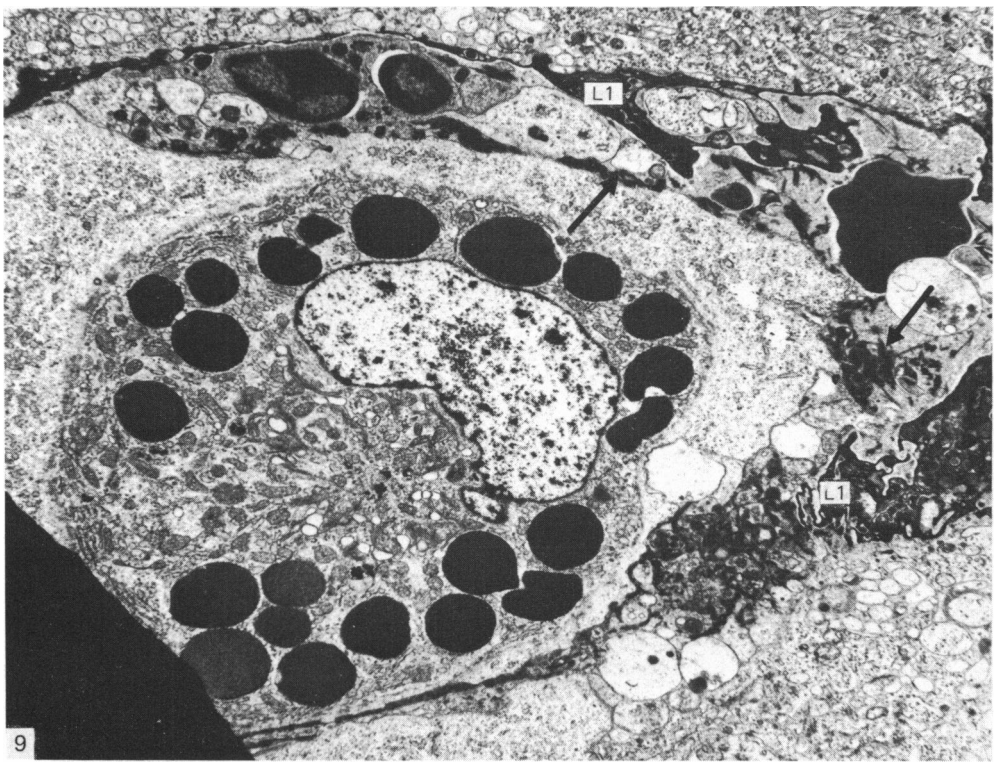


Fig. 8. Part of a healthy GMG cell (G) is shown lying adjacent to a healthy layer 1 trophoblast cell (L1). ×4300.

Fig. 9. A healthy GMG cell is shown lying adjacent to an electron-dense, degenerate layer 1 trophoblast cell (L1). Some fibrous material (arrow) is present in a space between the two cells. ×4800.

contained pale staining cytoplasm and large euchromatic nuclei. However, some of the trophoblast cells which were lying adjacent to GMG cells had a very densely stained nucleus and cytoplasm. These densely stained trophoblast cells were found lying adjacent both to GMG cells which appeared healthy and to those which appeared degenerate (Figs. 2, 3). A GMG cell was found next to a densely stained trophoblast cell as early as Day 10 of pregnancy (Fig. 5; Table 1) when the great majority of the sections of implantation sites showed invasion of the chorionic lamina by maternal blood spaces. Some degenerate GMG cells were found that were not associated with densely staining trophoblast cells (Fig. 4; Table 1).

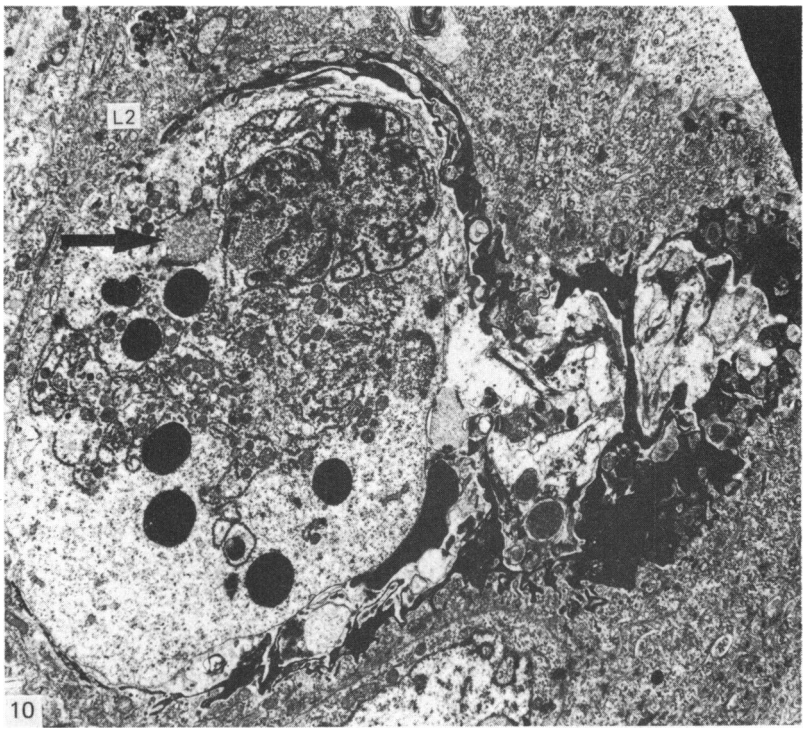
In some sections, one or two densely stained trophoblast cells were found which were not lying adjacent to a GMG cell. The cellular associations of 67 such trophoblast cells were examined in serial sections and in 63 cases a GMG cell was found in a neighbouring section (Fig. 6). The association of GMG cells with densely stained trophoblast cells was not limited to a 1:1 ratio (Figs. 2, 6).

With the electron microscope it was confirmed (see Enders, 1965) that the lumina of the maternal blood spaces and the fetal capillaries of the labyrinthine placenta were separated from each other by a barrier consisting of three trophoblastic layers and the fetal endothelium (Fig. 7). Layer 1 of the trophoblast was palely stained cytotrophoblast and lined the maternal blood spaces. Layer 2 trophoblast was syncytial in nature and usually appeared as the most electron-dense layer. Layer 3 trophoblast was also syncytial. A typical endothelium lined the fetal capillaries. The adjacent layers of the cellular barrier were generally closely apposed to each other.

Most of the GMG cells in the labyrinth appeared healthy and were similar to the healthy GMG cells found in the decidua basalis (Stewart & Peel, 1977). The large electron-dense granules with their distinctive membranous 'caps' were often arranged around a centrally orientated group of Golgi bodies. Mitochondria, granular endoplasmic reticulum and glycogen were usually found in the cytoplasm. All the healthy GMG cells appeared to be lying within the lumen of a maternal blood space and were often closely apposed to the layer 1 trophoblast (Figs. 8, 9). However, no cell junctions, such as desmosomes or tight junctions, were found between these two cell types.

Some GMG cells were found which showed early degenerative changes; the nuclei were irregular in shape and contained areas of condensed chromatin, the cytoplasmic organelles often appeared disorganised and sometimes one or two of the cytoplasmic granules were disrupted (Fig. 10) with loss of the matrix into the cytoplasm. Other GMG cells were found which appeared to be in a more advanced degenerative state. The nucleus of these cells was condensed or disrupted and the cytoplasm consisted largely of electron-dense granular material in which the usual cytoplasmic organelles could not always be identified (Fig. 11). Some of the typical cytoplasmic granules were still present but other granules were often irregular in shape and sometimes disrupted. In many cases the cell membrane was discontinuous or had disappeared.

The majority of layer 1 trophoblast cells were palely stained (Figs. 7, 8), but some had a more electron-dense cytoplasm (Figs. 9, 10). The nuclei in these electron-dense cells appeared smaller, more irregular in shape and more electron-dense with increased electron density of the cytoplasm. Some of these electron-dense, apparently degenerate, cells were so amorphous that it was difficult to identify a nucleus or any other organelle in the plane of section (Fig. 12). Sometimes a large space was found between electron-dense layer 1 trophoblast cells and the underlying layer 2 tropho-



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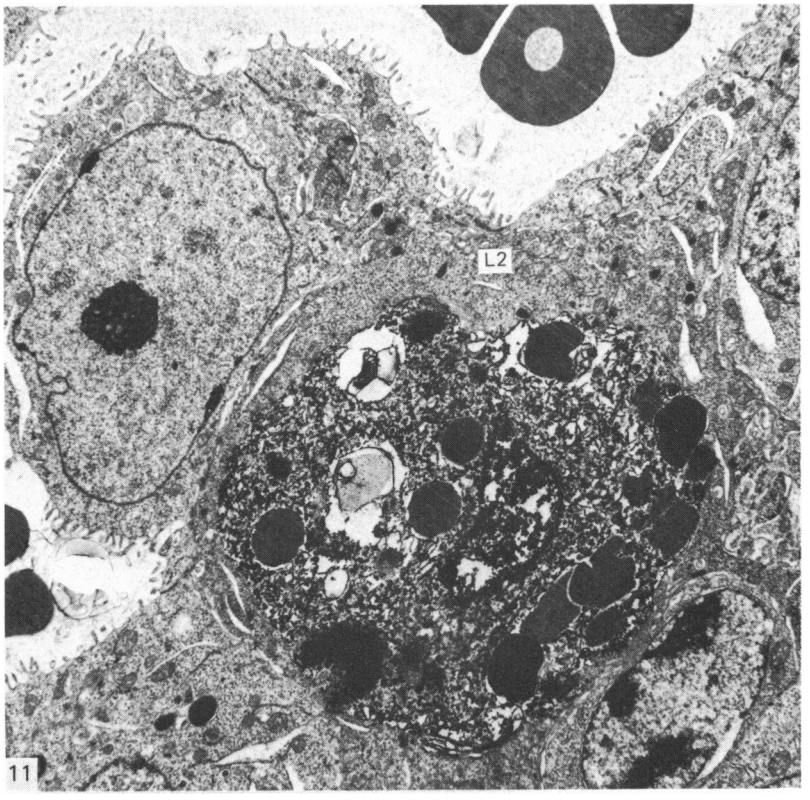

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blast (Fig. 13), with desmosomes providing only isolated points of attachment between the two layers. At other times, layer 1 trophoblast formed an incomplete layer so that part of the layer 2 trophoblast was lying immediately adjacent to the lumen of the maternal blood space (Fig. 10).

The associations seen with the light microscope, between GMG cells and layer 1 trophoblast cells, were confirmed with the electron microscope. Healthy GMG cells were found within the lumina of maternal blood spaces lined by palely stained layer 1 trophoblast cells (Fig. 8). In other cases, apparently healthy GMG cells were found adjacent to electron-dense trophoblast cells (Fig. 9); when this occurred, the electron-dense layer 1 was usually complete. GMG cells with some degenerative changes were found associated with electron-dense layer 1 trophoblast, both when the trophoblast formed a complete layer lining the maternal blood space and when layer 1 trophoblast was incomplete (Fig. 10). When GMG cells were found with extensive degenerative changes, they were associated with an electron-dense layer 1 trophoblast which was usually incomplete or, as in some instances (Fig. 11), completely absent. The remains of the GMG cells were then in intimate contact with the layer 2 trophoblast. No GMG cells were found adjacent to layer 3 trophoblast or to fetal endothelium. It was apparent that the cellular associations found in this study, including those illustrated in Figures 9 to 11, could represent sequential changes in a degenerative process involving an interaction between GMG cells and layer 1 trophoblast.

Fibrous material was often found in the extracellular matrix between GMG cells lying in the maternal blood space of the labyrinth and electron-dense trophoblast cells (Figs. 9, 13). At high magnification, this fibrous material could be seen to have a periodicity indicative of fibrin (Fig. 14). Sometimes large bundles of fibrin fibres were found which enmeshed platelets as well as erythrocytes and an occasional leucocyte. Some of the platelets had the spherical, empty appearance of activated platelets. Occasionally fibrin fibres were found in the space between layer 1 and layer 2 trophoblast when the electron-dense layer 1 trophoblast was separated from layer 2 trophoblast.

DISCUSSION

A close spatiotemporal relationship has been found between GMG cells and densely stained layer 1 trophoblast cells in the labyrinthine placenta. This relationship is evident from the earliest phase of labyrinthine development at Day 10 of pregnancy (Fig. 5) until late pregnancy (Table 1).

Dickson (1980) considered the possibility that mouse GMG cells, because of their large size, could become trapped in the maternal blood spaces of the labyrinth. However, GMG cells are sufficiently flexible to penetrate between the endothelial cells lining blood vessels in the decidua basalis and metrial gland (Stewart & Peel, 1978); Jbara & Stewart (1982) think that it is unlikely that the diameter of the maternal blood spaces in the labyrinth is small enough to prevent the passage of these

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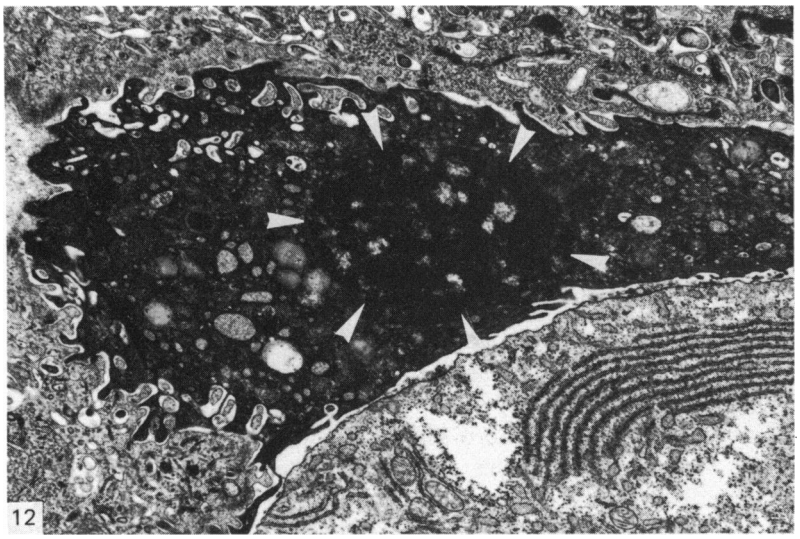
Fig. 10. A GMG cell with degenerative changes is shown lying adjacent to a degenerate layer 1 trophoblast cell. The nucleus of the GMG cell is irregular in shape and a disrupted granule (arrow) can be seen in the cytoplasm. Layer 1 trophoblast is incomplete so that part of the GMG cell is lying next to layer 2 trophoblast (L2). ×4500.

Fig. 11. A GMG cell is shown in an advanced stage of degeneration. The cytoplasm is composed of dense granular material and a cell membrane appears to be absent. No layer 1 trophoblast is present next to the GMG cell. L2, layer 2 trophoblast. ×3850.



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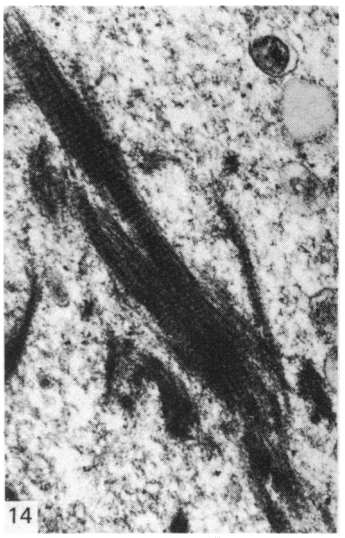

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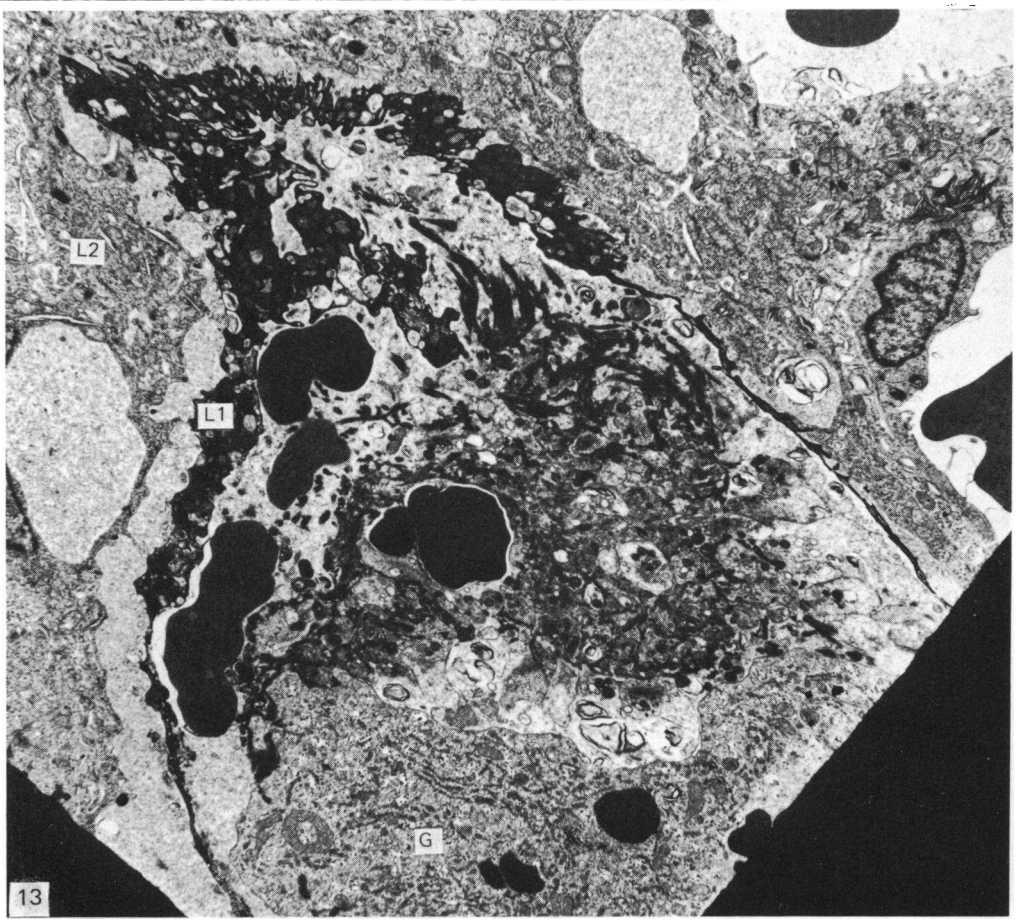

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cells. It is probable, therefore, that the GMG cells have a functional association with the densely stained degenerate layer 1 trophoblast cells which, from the cellular associations described, would appear to involve the localised degeneration and loss of layer 1 trophoblast cells as well as the degeneration of the GMG cells. It is tempting to suggest that the presence of blood clots at the sites of these interactions may be a mechanism to maintain this relationship. However, further studies are required in order to understand the dynamic nature of these associations.

Some GMG cells are found immediately adjacent to layer 2 trophoblast (Figs. 10, 11) and although no degenerative changes are found in the trophoblast, the GMG cells invariably show extensive degeneration. In general, GMG cells appear less degenerate than any degenerate layer 1 trophoblast cells with which they are associated and it is likely, therefore, that the association of GMG cells with layer 2 trophoblast is a result of an earlier loss of layer 1 trophoblast.

A large proportion of the GMG cells found in the maternal blood spaces of the labyrinth do not show any degenerative changes and are not associated with degenerate trophoblast cells. Some of these GMG and trophoblast cells may have been at such an early stage of their interaction that no structural evidence of degenerative change had developed. More detailed studies of the association between healthy GMG cells and healthy layer 1 trophoblast cells are in progress in an attempt to define any changes which occur during the earliest stages of the interaction between GMG cells and layer 1 trophoblast cells. Other healthy GMG cells may include those still in transit at the time of death, either to interact with trophoblast elsewhere in the labyrinth or perhaps to pass through the labyrinth to become part of the population of GMG cells that enter blood vessels which drain the uterus (Stewart & Peel, 1978).

Some GMG cells which are associated with degenerate trophoblast cells contain disrupted cytoplasmic granules. The granules are a prominent feature of GMG cells, and may be expected to have a role in their functional activity. However, the involvement of the granules in the interaction between GMG cells and layer 1 trophoblast cells is not clear. The active constituents of the granule matrix have not been determined, although a number of hydrolytic enzymes have been identified in the granules of rat GMG cells (Bulmer, 1968). Release of such enzymes following granule lysis could contribute to the degenerative changes found in some labyrinthine GMG cells.

The localisation of cytoplasmic immunoglobulin G in GMG cells of the pregnant rat uterus (Bulmer & Peel, 1977; Mitchell, Craggs & Peel, 1980) and the establishment of the bone marrow origin of GMG cells (Peel *et al.* 1983) has implicated these cells in the immunology of pregnancy. The maternofetal interface of the maternal blood spaces of the labyrinthine placenta is a site where cells involved in the immunology of pregnancy could be active. The GMG cells in the labyrinthine placenta are found in greatest numbers during the period of active growth and in greater numbers in

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Fig. 12. An electron-dense layer 1 trophoblast cell. It is difficult to identify the organelles but the darker area (delineated by arrowheads) in the centre of the cell is probably the remains of the nucleus. × 7300.

Fig. 13. The electron-dense layer 1 trophoblast cell (L1) has, in places, become separated from the underlying layer 2 trophoblast (L2). A mass of fibrous material can be seen between the GMG cell (G) and the layer 1 trophoblast. × 4800.

Fig. 14. A high magnification micrograph of the fibrous material found in association with GMG cells and degenerate layer 1 trophoblast cells. The fibres have a periodicity indicative of fibrin. × 47850.

outbred than in corresponding inbred pregnancies (Jbara & Stewart, 1982). However, as many GMG cells appear to lie freely within the lumina of the maternal blood spaces it may be of more relevance to establish the frequency of interaction between GMG cells and layer 1 trophoblast cells in inbred and outbred pregnancies. In addition, the factors which induce the migration of GMG cells into the blood vessels of the metrial gland and decidua basalis and the factors which are involved in initiating the interaction between these GMG cells and labyrinthine trophoblast must be determined. A deeper understanding of the antigenic determinants expressed on the surface of both normal layer 1 trophoblast cells, and those which show degenerative changes, may assist in resolving the latter problem in particular.

SUMMARY

A study has been made, using light and electron microscopy, of GMG cells in the labyrinthine placenta of the mouse. GMG cells were found lying within the lumina of the maternal blood spaces of the labyrinth. Some of the trophoblast cells lining the maternal blood spaces showed degenerative changes and GMG cells were usually found close to these degenerate trophoblast cells. Sometimes the GMG cells also showed degenerative changes. The observations suggest that an interaction may take place between GMG cells and layer 1 trophoblast cells and support the idea that the labyrinthine placenta is a site of functional significance to GMG cells.

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