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The ultrastructure of pig trophoblast transplanted to an ectopic site in the uterine wall

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INTRODUCTION

As previously reported (Samuel, 1971), pig trophoblast survives for some time, and develops invasive properties, after transplantation to a variety of ectopic sites, including the uterine wall. In such sites, the cells tend to aggregate and coalesce into syncytial masses. In the present study, trophoblast tissues have been transplanted to the uterine wall and examined by electron microscopy after an interval of four or seven days. This account will be limited to an examination of two aspects of the morphological changes observed: the syncytial transformation and the interaction between the trophoblast and the surrounding uterine tissues.

MATERIAL AND METHODS

Sows were given 500 i.u. HCG (Lutormone; Burroughs Wellcome) by intramuscular injection late in the oestrous cycle and were mated, at the ensuing oestrus, with boars of known fertility. Ovulation was assumed to occur 44–48 hours after this injection (Polge, 1967) and eggs were recovered and transplanted under general anaesthesia 172 ± 4 hours after the calculated time of fertilization. At this stage the blastocyst is still within the zona pellucida. It is about 100 μm in diameter, comprises some 300 cells, and has reached a stage in its development comparable with the blastocysts of mice, rats and guinea-pigs as used by other workers – the trophoblast of which is normally invasive. Such blastocysts are relatively easy to handle, and provide a substantial number of cells, consisting almost exclusively of trophoblast and inner cell mass.

Anaesthesia was induced by the injection of sodium pentobarbitone (Nembutal; Abbott) into an ear vein, and maintained with halothane (fluothane; I.C.I.) in a closed-circuit system. The uterus was exposed by a midline incision. Sterile glass cannulae were inserted into the cervical and ovarian ends of each horn separately, and approximately 150 ml sterile saline was 'milked' through into a collecting vessel. Using a Pasteur pipette, the blastocysts were transferred singly through the serosal (peritoneal) surface of the uterine wall into small pockets between the muscle layers or within the mucosa. In some instances a small pledget of surgical gel (Sterispon; Allen & Hanbury) was inserted to prevent loss of the eggs, and in all cases the incision was closed with a fine gut suture.

In the first experiments, an interval of seven days was allowed between the time

of transplantation and recovery of the tissues for examination. This interval was chosen because the blastocyst, if left in its natural habitat within the uterine lumen, would normally have undergone a considerable increase in cell number, with relatively little further embryogenic differentiation. However, since the trophoblast in the ectopic transplants had undergone considerable alteration, and in places had become syncytially transformed, the interval between transplantation and recovery was shortened to four days in a subsequent experiment to provide information about the antecedent changes.

At autopsy, explants were located and removed together with the surrounding tissues. Some were fixed in Bouin's fluid and embedded in paraffin wax for light microscopy, while others were fixed in glutaraldehyde buffered in phosphate or collidine, post-fixed in 1 % osmium tetroxide, and embedded in Araldite (CIBA) for examination under the electron microscope. These blocks were scanned in 0·5 μm sections stained with 1 % toluidine blue in 1 % borax; thin sections, mounted on uncoated copper grids and stained sequentially with uranyl acetate and lead citrate, were examined in a JEM T7 electron microscope.

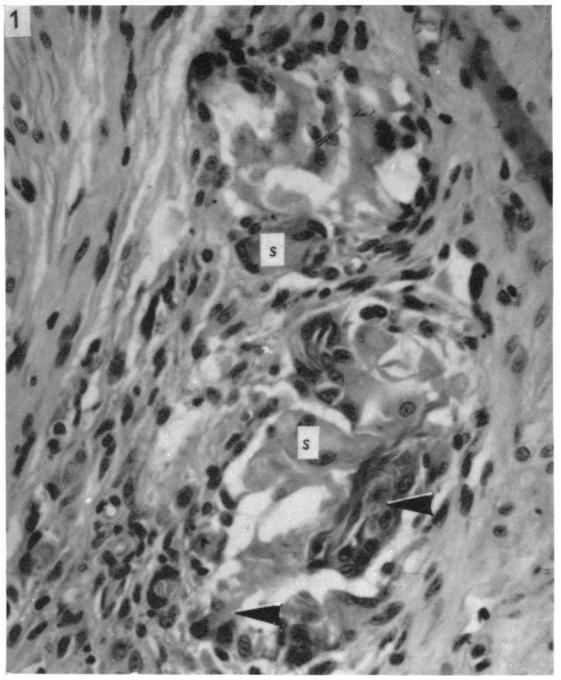
RESULTS

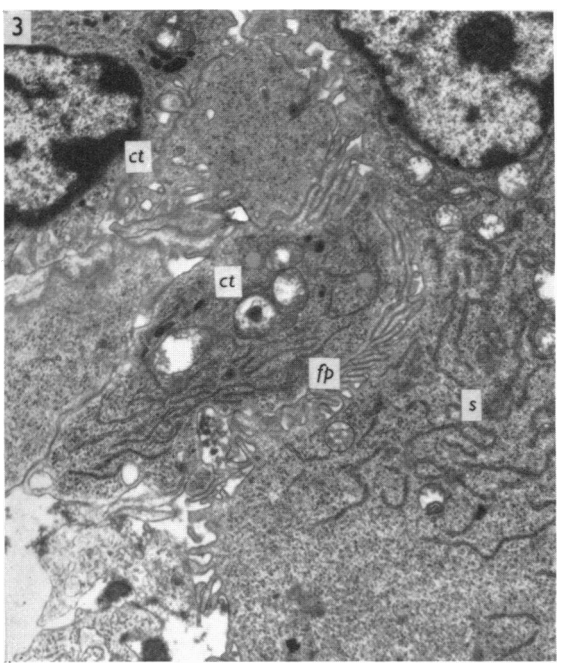
Formation of trophoblastic syncytium

After four days in the ectopic sites, whether between the muscle layers or within the mucosa, the blastocyst has undergone some important changes. The zona pellucida is lost, and no remnant of it has been identified in any of the explants. The hollow spherical form is also lost and it is probable that the surviving cells are exclusively of trophoblastic origin, no remnants of the inner cell mass being identifiable. Under the light microscope the cells appear aggregated into clumps, with pale and vesicular nuclei and markedly eosinophilic cytoplasm (Fig. 1, arrows). Part of such an aggregation, viewed under the electron microscope, is shown in Fig. 2. The cells (*ct*) show many of the characteristics of normal cytotrophoblast; there are few organelles except large round mitochondria, and the plasmalemma is produced into long frond-like processes (*fp*) reminiscent of the pre-attachment stages in this species.

Syncytial masses are also observed, formed presumably by the coalescence of the aggregated cells, and by the incorporation of further cells (Fig. 1, *s*). Nuclear division unaccompanied by cytokinesis conceivably contributes to the process, but no evidence of karyokinesis in the syncytium has been seen. Fig. 3 shows the margin of a syncytial mass (*s*) to which several cells (*ct*) are closely attached, the frond-like processes (*fp*) of the adjacent surfaces being intimately intertwined. The characteristics of the individual cells are retained for a time after the plasma membranes have been lost, and Fig. 4 shows an early stage in the formation of the syncytium in which several features of the cytotrophoblast have been retained. There is a band of less dense 'syncytioplasm' (arrow) around the perimeter of the mass, which is free of organelles and which is wider where one syncytial mass abuts onto another. The presence of the band may be related to the fusion of the adjoining masses.

Although, under the light microscope, these groups of cells and areas of syncytium somewhat resemble the 'foreign-body giant cells' produced at the site of trauma,





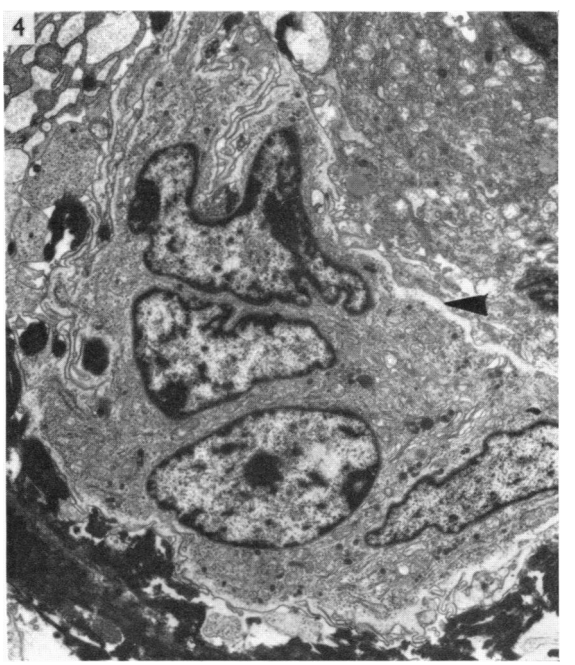


Fig. 1. Light micrograph of the ectopic trophoblast in the wall of the uterus. × 200, haematoxylin and eosin.

Fig. 2. Electron micrograph of a group of trophoblast cells. × 1000.

Fig. 3. Cytotrophoblast closely approximated to the syncytium. × 15000.

Fig. 4. An early stage in the growth of the syncytium. × 1000.

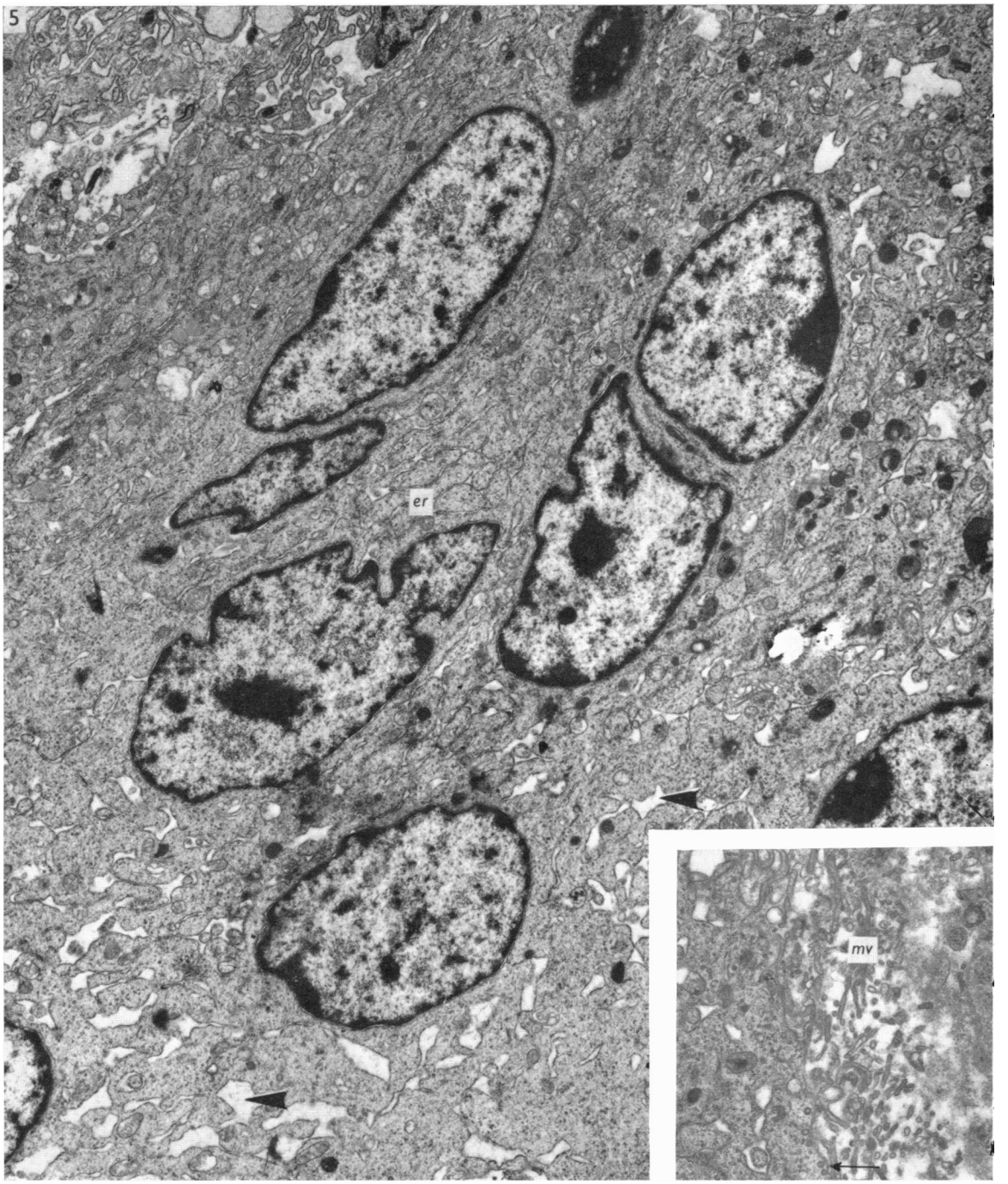
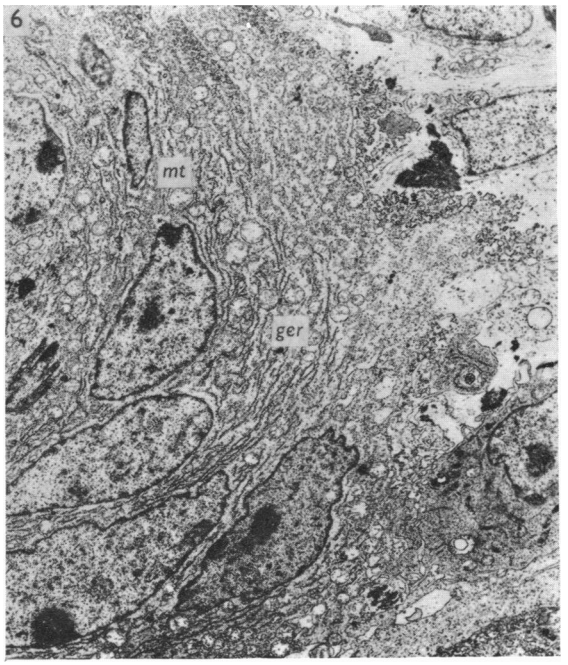
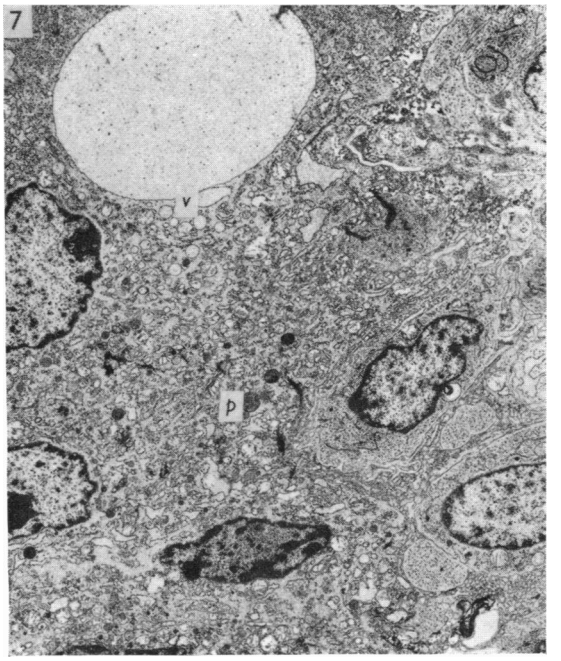


Fig. 5. Typical syncytium from the four-day explant. ×4500. Inset: border of the syncytium ×6500.





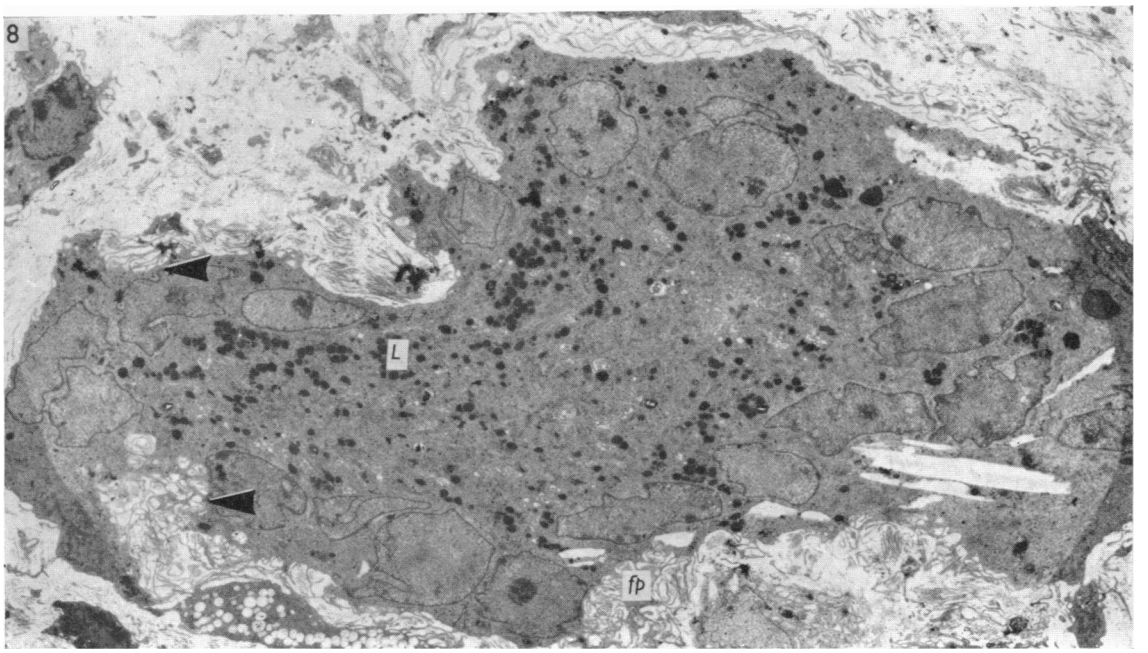


Fig. 6. Syncytium showing extensive development of granular endoplasmic reticulum. ×2000.

Fig. 7. Syncytium showing vesicular structures. ×2000.

Fig. 8. Mature syncytium from a seven-day implant. ×900.

they are readily distinguishable when examined by the electron microscope or by a polarizing microscope.

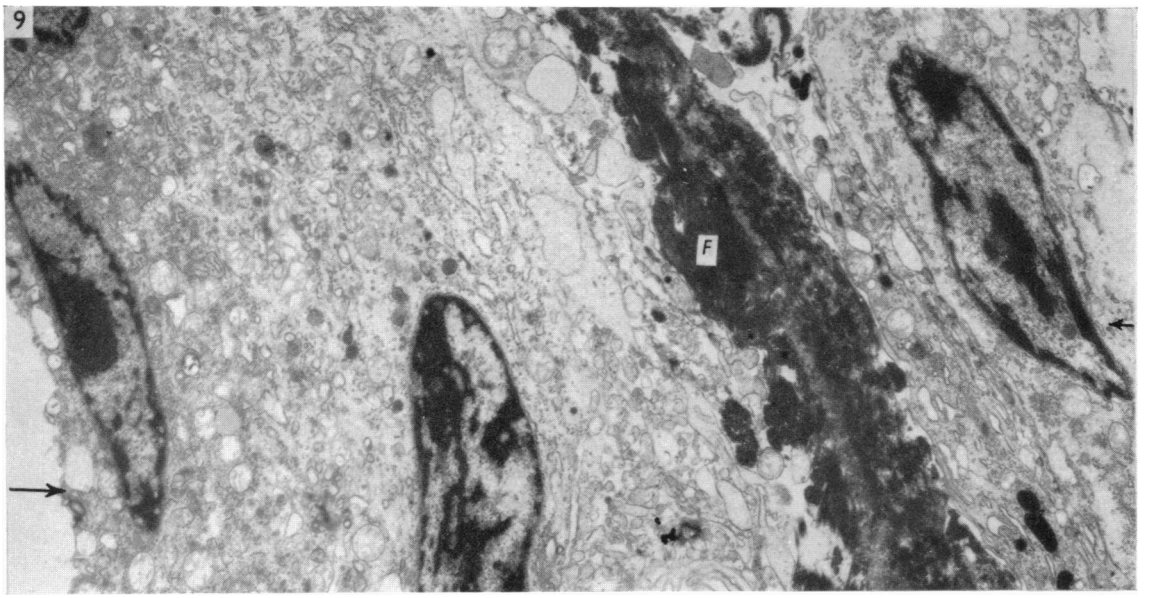
The character of the syncytium in the four-day explant varies considerably from place to place. The commonest form is that shown in Fig. 5, in which the nuclei are large, often irregular in outline, and contain prominent nucleoli and a concentration of chromatin around the inner face of the nuclear membrane. The endoplasmic reticulum (*er*), which occupies a large part of the syncytioplasm, contains many distended cisternae (arrows) filled with a flocculent material. The margin of the syncytium is extremely irregular (inset, Fig. 5) and is beset with long microvilli (*mv*). There are well defined vesicles (arrow) near the surface in association with the microvilli and doubtless involved in pinocytosis. Much of the endoplasmic reticulum is free of ribosomes, but in places the syncytioplasm is packed with short lengths of granular endoplasmic reticulum (Fig. 6, *ger*), and in these regions the mitochondria (*mt*) are slightly swollen. Another form assumed by the four-day graft is shown in Fig. 7. Here the syncytioplasm has a spongy appearance owing to the presence of large numbers of vesicles (*v*) of various sizes, each bounded by unit membrane. There are also small vesicles containing electron-dense material (*p*); the membranous contents of some of these suggest that they are phagosomes.

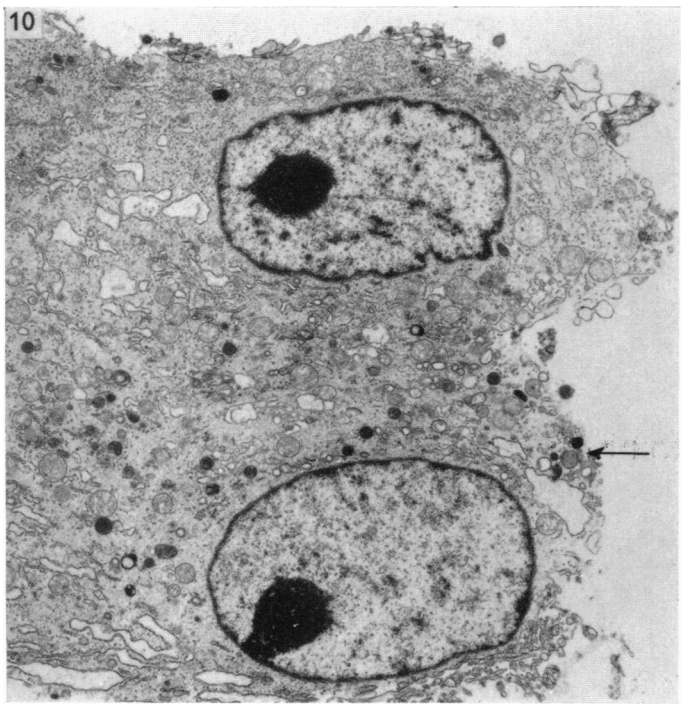
By the seventh day after transplantation, the tissue is much more uniform and the margin of the masses has become much less dissected over most of its extent (Fig. 8). The frond-like processes (*fp*) appear only over limited areas of the surface, and some of these areas are drawn into the bulk of the syncytioplasm so that they appear in sections to be enclosed within it (Fig. 8, arrows). Lysosomes (*L*) have become much more numerous, the whole mass has a rather dense appearance, and there is a noticeable reduction of endoplasmic reticulum. All of these features are indicative of an imminent degeneration.

Interactions between the trophoblast and the uterine tissues

Active exchange between the syncytium and the surrounding cells and tissue fluids is evidenced by the appearance of the syncytial surface. The frond-like microvilli or cell processes, already referred to, are typical of those encountered in tissues to which an absorptive function can be ascribed; in places small 'coated' vesicles can be seen near the surface (Fig. 5, inset, arrow).

The response of the uterine tissue to the presence of the trophoblast is more difficult to define. Areas of the syncytium where the plasmalemma is discontinouus (Figs. 9, 10, arrows), suggest that dissolution may begin even within four days, and this may result from the activity of uterine stromal cells and leucocytes. On the other hand, such cells (Fig. 11, *L*) often appear to be engulfed by the syncytium (*s*) or to undergo degeneration in its vicinity (Fig. 12, *L*). Three distinct kinds of extracellular material are found in association with the trophoblast. Collagen, identified by its characteristic periodicity when examined at high magnification, is present in large quantities in the uterine stroma (Fig. 13, *c*) and appears to constitute a barrier to the further invasion of the trophoblast. In some places fibroblasts (*fb*) may be seen close to the syncytium, and the appearance of their endoplasmic reticulum suggests that they may be implicated in an increase in the amount of collagen associated with the explanted trophoblast. A second type of extracellular material is that illustrated





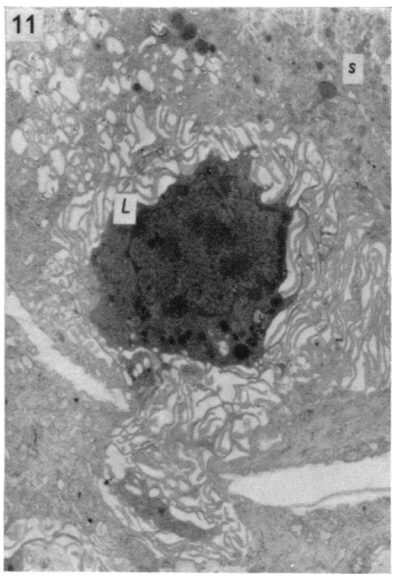
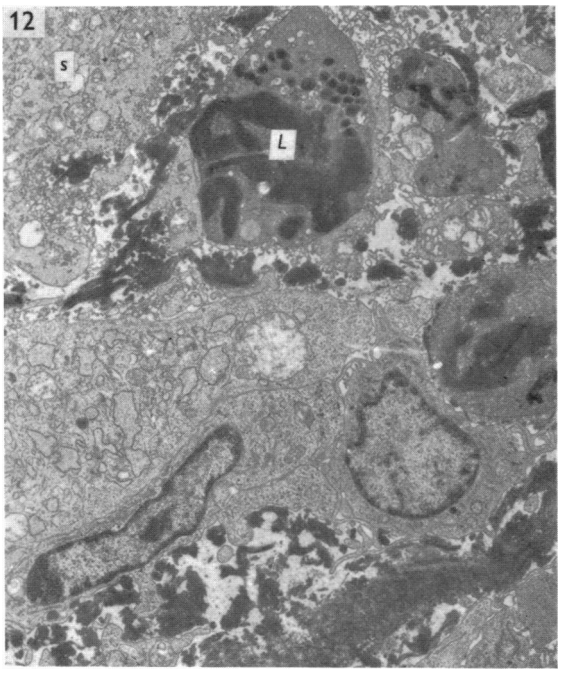
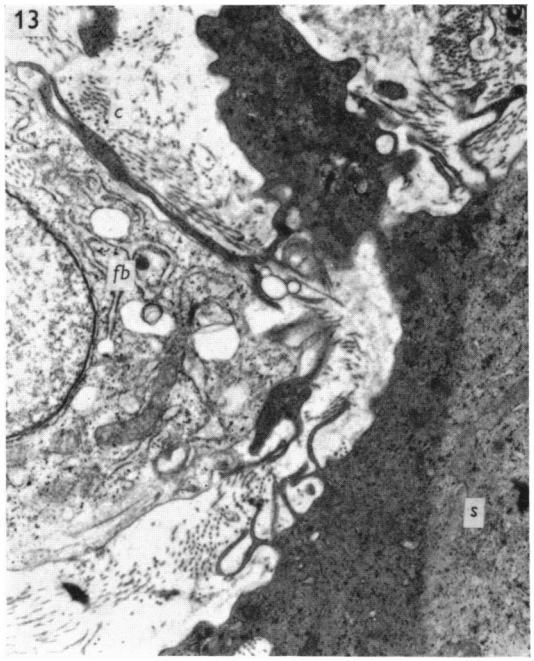


Fig. 9. Degenerating syncytium contained (on right of photograph) by a uterine product believed to be fibrin. ×1500.

Fig. 10. Syncytium lacking a complete plasma membrane. ×2000.

Fig. 11. Maternal leucocyte engulfed by the syncytium (7 days). ×900.





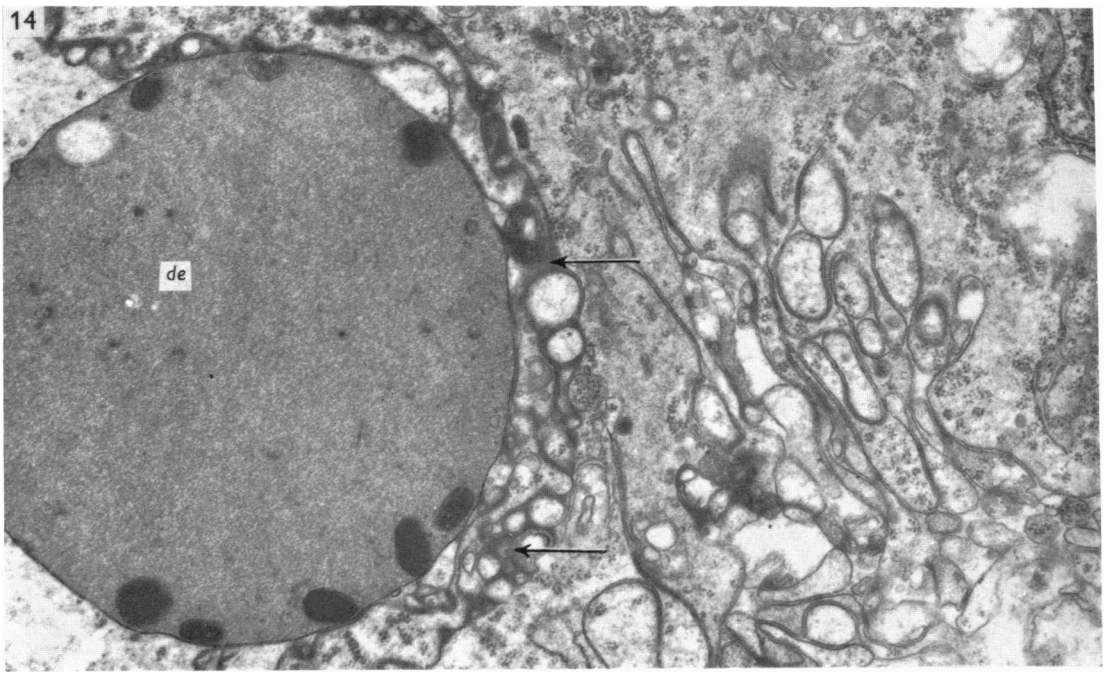


Fig. 12. Leucocyte undergoing degeneration in the vicinity of the syncytium (4 days). ×900.

Fig. 13. Fibroblast in close apposition to the syncytium (7 days). ×2000.

Fig. 14. Border of the syncytium from four-day explant showing dark deposit, possibly sialomucin, around the microvilli. ×5500.

in Fig. 9, where there is a dense flocculent and fibrous material (*F*) in close proximity to the syncytium. From its appearance in electron micrographs, this material appears to be fibrin; its origin is not clear, but it may be derived from extravasated blood released during the insertion of the graft. A third type of deposit is shown in Fig. 14 (arrows); this consists of an electron-dense deposit whose nature we have not been able to determine, associated with parts of the syncytial border where the margin is greatly folded and perhaps even broken, to give the globular appearance seen in the photograph. The large round mass to the left of this photograph is thought to be a degenerate erythrocyte (*de*).

DISCUSSION

In species where the trophoblast is normally invasive, part of it invariably undergoes a syncytial transformation (Amoroso, 1961). However, in such a placenta the continued advance of the trophoblastic (chorionic) villi must depend upon the increase of the syncytial mass by proliferation from the associated cytotrophoblast. In the ectopic transplants described in the present investigation, cytotrophoblast was only discernible for a brief period after transplantation. The proliferated tissue did not attain the compact cellular character normally associated with its development within the uterine lumen, but became dispersed in syncytial fronds quite unlike any form seen during the normal development of the blastocyst. Hence it may be presumed that the uterine epithelium has an essential role in controlling the normal course of the development of the trophoblast of the pig, and at the same time constrains its invasive properties and its syncytial transformation.

In the ectopic sites, various stages have been described in relation to the growth and ageing of the transplanted trophoblast. After four days (Fig. 4) its development by coalescence of individual cells parallels that described in human chorionic villi (Boyd & Hamilton, 1966). Its appearance in this figure, and in Figs. 6 and 7, is indicative of a tissue engaged in active metabolic exchange with the surrounding tissues, and in which there is evidence of considerable biosynthetic activity. The trophoblast may thus contribute to the extracellular material seen throughout the explants. On the other hand, the trophoblast of many species is known to synthesize steroid hormones, and that of the pig secretes oestrogen in increasing quantities from mid-gestation (Challis, personal communication). In view of the precocity of the transplanted trophoblast, synthesis of this kind is not inconceivable in ectopic sites.

Differences in the appearance of the four-day syncytium (cf. Figs. 4 and 9, and Figs. 6 and 7 respectively) may represent an ageing and degenerative process, or may be the result of an immunological response of the host tissues. The characteristics shown in Fig. 9 are indicative of ageing; the nuclei are pyknotic, the syncytioplasm is disorganized, and only fragments of the endoplasmic reticulum and Golgi complex are visible. Often the mitochondria are swollen, and at a number of points on the surface of the syncytium the plasma membrane is discontinuous. Similar degenerative features have been described in the late placenta of the rat (Jollie, 1965) and in ectopic transplants of the mouse placenta (Kirby & Malhotra, 1964). The presence of large numbers of lysosomes in the seven-day transplant of the pig trophoblast, together with vesicles containing remnants of membranes

(phagosomes) is indicative of active phagocytosis comparable with that described in the normal development of the placenta of the rat (Tachi, Tachi & Lindner, 1970), rabbit (Larsen, 1962), mouse (Mulnard, 1967) and human (Wislocki & Bennett, 1943).

The response of the uterine stroma to the presence of the trophoblast was not marked, but it must be remembered that the amount of tissue transplanted was minute in relation to the mass of the uterus. The micrographs nevertheless give some indication of an increased amount of collagen deposited in the vicinity of the trophoblast. There also appeared to be an increase in the local concentration of monocytes and leucocytes between the fourth and seventh days. This reaction resembled the beginnings of a foreign tissue reaction but was not accompanied by an accumulation of platelets and was not comparable in scale with the decidual reaction in the primate placenta. It did not at all resemble the 'decidual cell reaction' of the rodent placenta and in these experiments the endometrial stroma showed no such response (cellular hypertrophy and glycogen accumulation) to the trophoblast artificially implanted within it. It is not clear to what extent the syncytial transformation of the ectopic transplant is related to its invasiveness, or whether it represents a defensive reaction to the activity of the uterine tissues. It is clear that the cells of the transplanted trophoblast failed to continue to divide mitotically, that they lost their 'embryonic' appearance, underwent a syncytial transformation and precocious ultrastructural differentiation, and gave marked indications of metabolic activity over a period of about a week, after which they degenerated.

SUMMARY

Blastocysts recovered from the uterine lumen of pigs seven days after mating were transferred to ectopic sites within the uterine wall. Tissue was recovered either four or seven days later, and the condition of the trophoblast cells was studied by light and electron microscopy. Only trophoblast cells appeared to survive; they first aggregated into clumps and later fused to form syncytial masses, with an extremely irregular margin beset with long microvilli. The syncytioplasm contained considerable amounts of both smooth and granular endoplasmic reticulum and, in places, numerous vacuoles which gave it a spongy appearance. By the seventh day, there was evidence of degeneration in the trophoblastic masses, in which lysosomes had become very prominent. The uterine stromal cells appeared to react to the presence of the trophoblast by an increased local production of collagen, and by the deposition of other non-cellular material. There appeared to be increased activity of elements of the reticulo-endothelial system within the uterine stroma at the site of the implant, resembling the beginnings of a foreign tissue reaction; there was no evidence of a decidual cell reaction.

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