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Structure of trophoblast papillae on the sheep conceptus at implantation

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INTRODUCTION

It is vitally necessary for the developing embryo of viviparous mammalian species to establish an intimate and firm contact with the uterine food source. In some species, implantation involves invasion of the uterine tissue by the embryonic trophectoderm (e.g. rat, dog, cat) whereas in others a close apposition of epithelia is sufficient (e.g. pig, horse) (Amoroso, 1952). In the sheep, implantation is characterised by the formation of a syncytium in place of the original cellular caruncular uterine epithelium after apposition of the fetal trophectoderm (Amoroso, 1952; Boshier, 1969; Wooding & Staples, 1981).

This paper presents evidence for the presence of columns of cells ('papillae') on the external surface of the embryonic region of the sheep conceptus between days 13–18 of pregnancy. The papillae are formed by the growth of trophectoderm cells down into the lumina of the uterine glands. The papillae develop well before the uterine epithelium is replaced by a syncytium.

It is suggested that the papillae may have an important role in immobilising the chorion against the uterine epithelium. This would facilitate the interaction between fetal and maternal surfaces which leads to the initiation of a layer of syncytium characteristic of the ovine cotyledonary placenta.

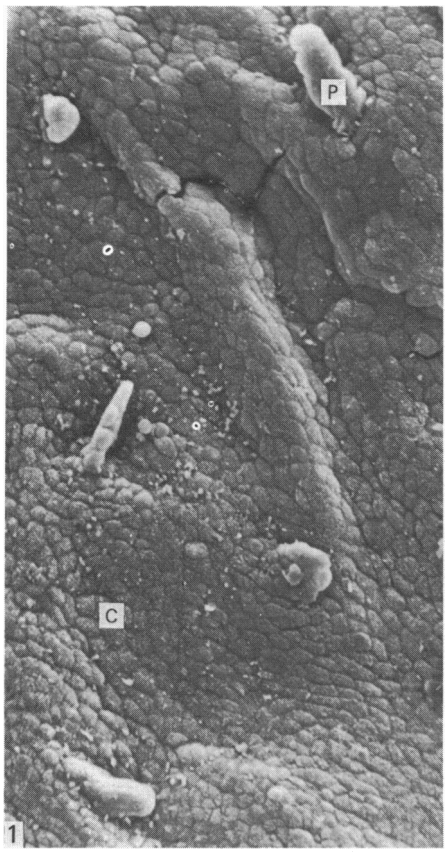
METHODS AND MATERIALS

Collection and fixation of tissues

For studies of the nature of the trophectoderm surface of isolated peri-implantation conceptuses, these were fixed and recovered by gently flushing 2·5 % glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2) through the lumen of the excised uterus of Corriedale or Clun Forest sheep killed at between 11 and 18 days after mating. The conceptuses were examined under a dissecting microscope at × 4 to 20 magnification to establish the distribution of the papillae. Selected regions of the blastocyst were then processed for electron microscopy (see below).

For studies of the location of placental structures *in vivo*, pregnant Soay or Clun Forest sheep were killed with pentobarbitone sodium 14–29 days after service. The uterus was immediately removed, the uterine arteries were cannulated and perfused with 4 % glutaraldehyde in 0·1 M phosphate buffer at pH 7·2 and containing 2 % sucrose.

Glutaraldehyde was also injected into the uterine lumen (1–2 ml/horn) after an initial 100 ml had been perfused through each uterine artery. Areas of apposition between blastocyst and uterine epithelium were dissected out and processed for electron microscopy (see below).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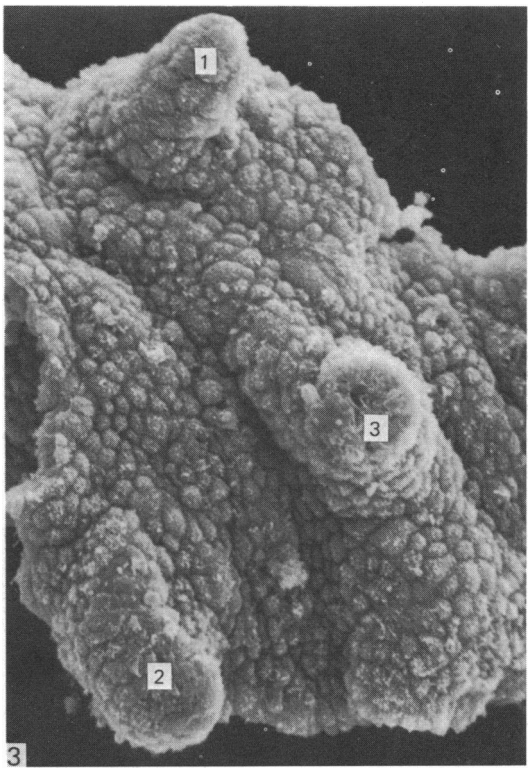
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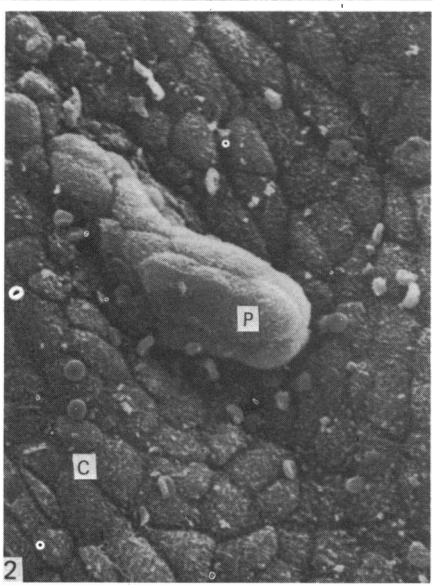
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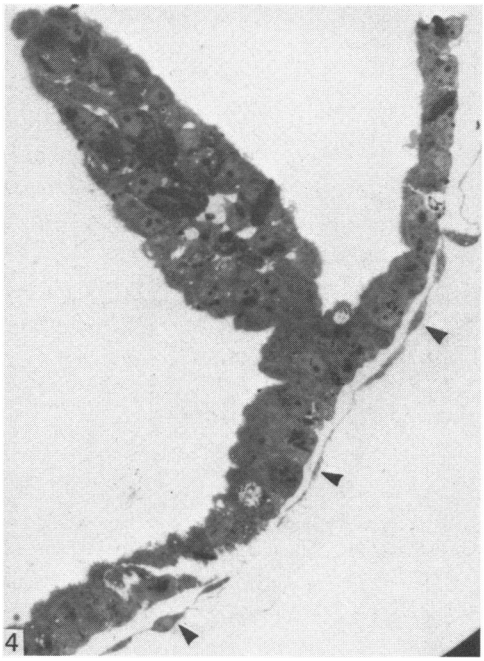
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Electron microscopy

Glutaraldehyde-fixed tissue was passed through phosphate buffer (30 minutes), 1 % osmium tetroxide in buffer at pH 7·2 (60 minutes) and 2 % aqueous uranyl acetate (30 minutes). The tissue was then dehydrated in alcohol (or acetone) and propylene oxide and embedded in Araldite or Epon resin. 0·5–1·0 μm sections were cut, mounted on glass slides and stained with hot 1 % toluidine blue in 1 % sodium borate, before being washed, dried and mounted in Araldite for light microscopy.

Because pieces of conceptus with papillae, embedded in Epon, were immediately available, the tissue was prepared for scanning microscopy by dissolving away the Epon in a solution of sodium methoxide and benzene (Mayor, Hampton & Rosario, 1961). The tissue was then critical point dried (from amyl acetate) and sputter coated.

RESULTS

The earliest stage of development at which papillae were observed on an isolated conceptus was at day 13 after service (Figs. 1, 2). This conceptus was 25 cm long. A second, nominally 13 day conceptus only 2 cm long, showed no papillae and none was seen on an 11 day spherical conceptus. The longer 13 day conceptus, two conceptuses at day 14, and one each at days 15 and 18 showed papillae limited to the central embryonic region and scattered apparently randomly on the surface (Figs. 1, 3). Between 50 and 200 papillae on the central two or three centimetres of each conceptus survived isolation. The irregular outline and fragility of the conceptuses made counting of the papillae difficult. Only two papillae could be found on one 16 day conceptus. At days 13 and 18 the papillae were of variable size and shape but were consistently longer and thicker by day 18. The number per unit area of trophectoderm could not be shown to alter with age.

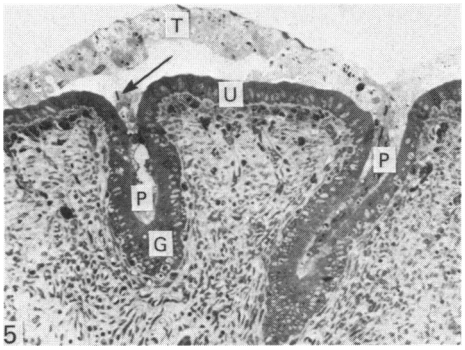
1 μm sections of plastic-embedded conceptuses showed that the papillae consisted of trophectoderm cells only, and that the endodermal cell layer did not participate in their formation (Fig. 4). Light and electron microscopy demonstrated that the thin endodermal layer was continuous across the base of the papilla (Figs. 4, 11), and that the basement membrane normally associated with the ectodermal cells was absent from the central core of the papilla. These observations clearly distinguished the papillae from those extensions of the conceptus which formed narrow folds in the uterine epithelium (Fig. 9). Although narrower than some papillae, the folds of the conceptus had endoderm and the basement membrane of the trophectoderm extending right down into the tip (Fig. 10).

Fig. 1. Isolated 13 days old sheep conceptus. Scanning electron micrograph of (outer) surface of the conceptus (C) showing five papillae (P) of various shapes and sizes ×180.

Fig. 2. Detail of Fig. 1. The cellular outlines and surface microvilli of the trophectoderm cells comprising the surface (C) and papilla (P) of the conceptus can be clearly distinguished. ×540.

Fig. 3. Isolated 18 days old sheep conceptus. Scanning electron micrograph of a small piece of the conceptus with large papillae (1, 2) and a third which has been broken off (3). ×310.

Fig. 4. From the same isolated conceptus as in Fig. 3. Light micrograph of a 1 μm plastic section of a papilla. Note that the endoderm (arrowheads) does not play any part in the papillary structure. ×520.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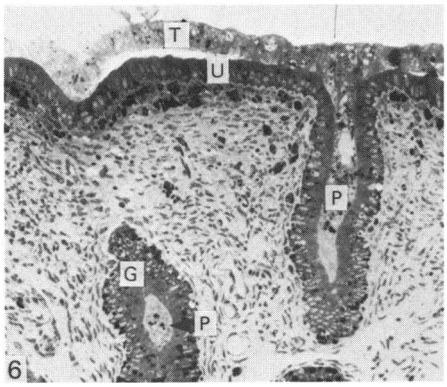
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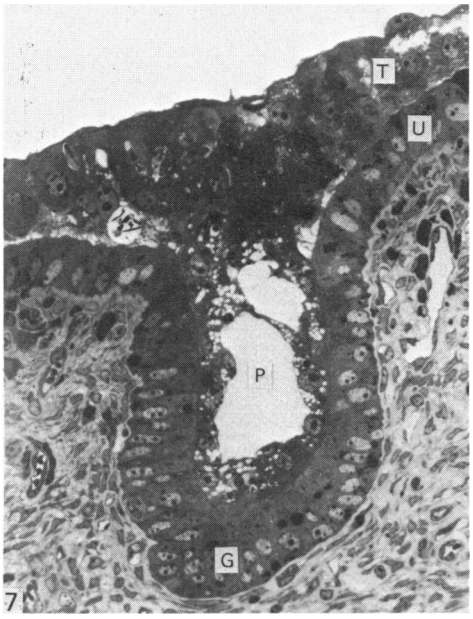
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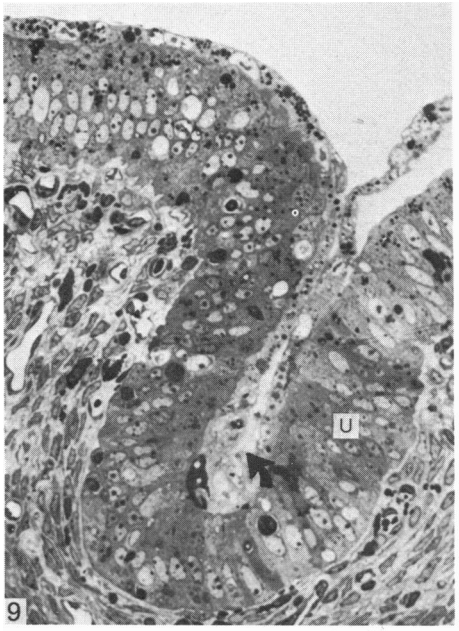
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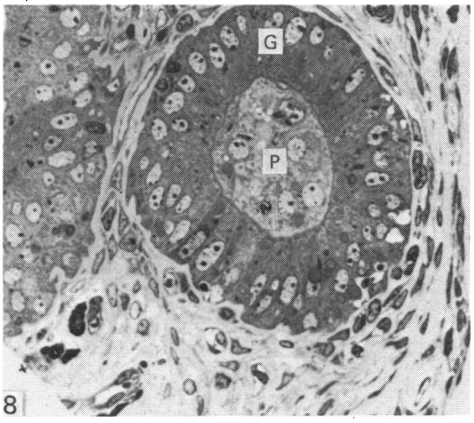
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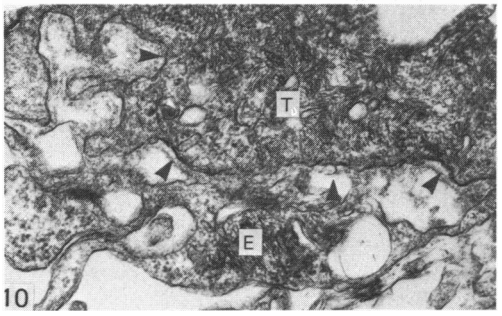
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In the pregnant uterus fixed by perfusion, contact between conceptus and endometrium was first seen at day 16 in the region of the embryonic disc. The uterine epithelium was intact and the lumen of each of the many glands was deeply penetrated by a cellular outgrowth of the conceptus trophectoderm (Figs. 5–8; 11–13). These outgrowths were similar in size and shape to the papillae on the isolated conceptus. Indications of papillae in the glands were found in specimens at 16, 17 and 19 days after service.

The cells forming the papilla were of very similar ultrastructure to those forming the rest of the trophectoderm and were linked with tight junctions close to their free surfaces (Fig. 12). They characteristically contained large membrane-bounded crystalline structures, large lipid droplets, numerous small mitochondria, scattered cisternae of rough endoplasmic reticulum, a small Golgi area and a large nucleus (Figs. 11, 12, 17, 19). Binucleate cells were observed in the papillae (Fig. 11) and occurred with increasing frequency elsewhere in the trophectoderm from day 16.

The microvilli on the apical surface of the glandular epithelium interdigitated to a variable extent with the microvilli of the papilla (Figs. 14, 15) as they did in other regions where the conceptus was apposed to uterine epithelium. There were always far more microvilli on the uterine epithelial surface than on the rather flattened cell apices of the papillae (Fig. 15).

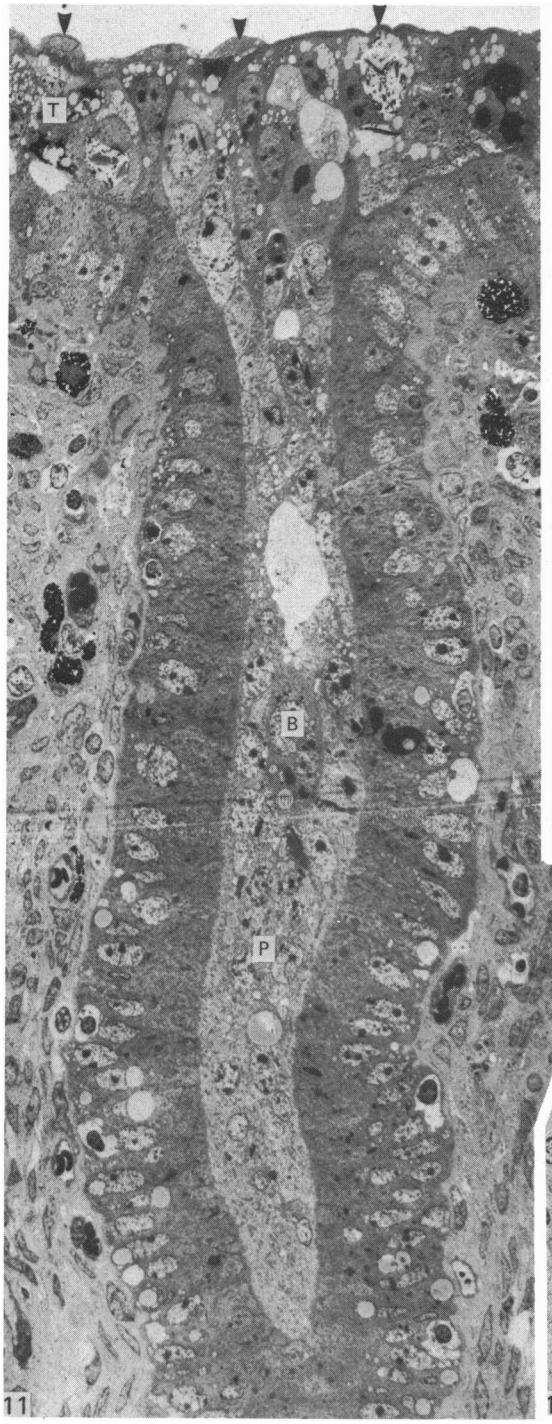
The cells of the papilla were usually packed together tightly in the lumen of the gland (Figs. 8, 12) but occasionally there were central intercellular spaces in the papilla (Figs. 7, 19). The papillary cells did not rest on a continuous basement membrane but some membrane remnants were evident (Fig. 18). There were tight junctions sealing the papillary cells together where they faced the glandular epithelium, which was apparently unaffected by the close apposition of the cell surfaces.

The papillae usually remained part of the conceptus and pulled out of the glandular lumina but occasionally broken-off stubs of papillae were seen in glands in areas where the conceptus had been loosened or lost during processing (Fig. 5). Fragments of membrane were occasionally seen adhering to the uterine epithelial microvilli where the conceptus had torn away locally (Figs. 16, 17). Between 17 and 19 days of pregnancy, evidence was found of degeneration at the tips of some papillae deep in the glands (Fig. 13). The lumina of other glands contained masses of pyknotic nuclei, loose crystalline material and lipid.

No papillae were found in the glands at or after 20 days of gestation.

Figs. 5–8. 16 days old sheep conceptus fixed *in utero*. Light micrographs of plastic sections showing solid (Figs. 5, 6, 8) and vacuolated (Figs. 5, 7) papillae (P) penetrating deep into the uterine glands (G). There is no evidence of trophoblast endoderm in any of the papillae. The trophectoderm (T) of the conceptus has separated (probably artifactually during processing) from the uterine epithelium (U) in places and one papilla (arrow in Fig. 5) has broken off from the trophectoderm. In the glands, the papillae show a very close apposition to the glandular epithelium in longitudinal (Figs. 5–7) and transverse (Fig. 8) sections. Fig. 5 × 130; Fig. 6 × 130; Fig. 7 × 525; Fig. 8 × 350.

Figs. 9–10. 17 days old sheep conceptus fixed *in utero*. Fig. 9 is a light micrograph of a plastic section showing penetration of a narrow fold in the uterine epithelium (U) by the conceptus. Both trophectoderm and endoderm are present in the fold. This is demonstrated by Fig. 10, an electron micrograph of the tip of the fold (at curved arrow, Fig. 9) showing trophectoderm (T) separated by a definite basement membrane (arrowheads) from the thin endoderm (E). Fig. 9 × 360; Fig. 10 × 22000.



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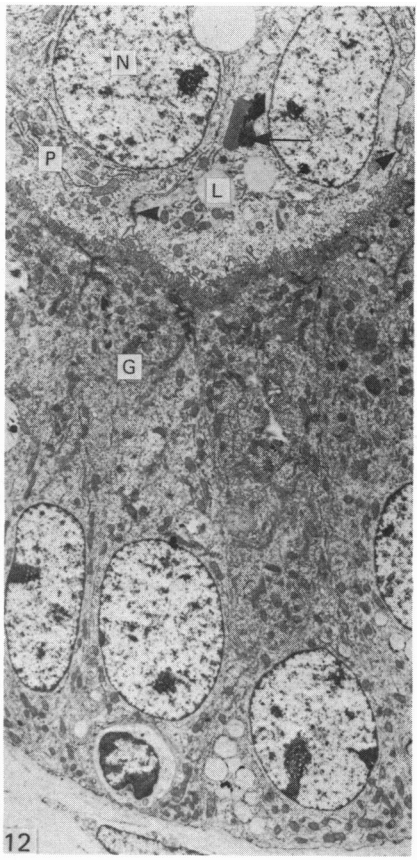

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DISCUSSION

The numerous outgrowths of trophectoderm which we have called 'papillae' were a constant feature of the embryonic region of elongated sheep conceptuses examined between 13 and 18 days of gestation, with one exception (at 16 days) on which only two papillae were found. In this case, it seemed unlikely that all the papillae had broken off during isolation, and the length, diameter and general appearance of that conceptus seemed within the normal ranges. The significance of this single exception must await further investigation.

Previous reports (Boshier, 1969; Wintenberger-Torres & Flechon, 1974) have made no mention of papillae, but they are too small to be seen readily with the naked eye and insufficiently frequent to appear consistently in anything other than serial sections. In a recent colloquium Flechon, Guillomot & Wintenberger-Torres (1978) have briefly noted the presence of protrusions on a 16 day conceptus, but no details were given. Because papillae are evident only between 13 and 20 days of pregnancy, the studies on the sheep blastocyst at earlier (Calarco & McLaren, 1976) or later stages (Davies & Wimsatt, 1966; Bjorkman, 1965) could not have detected them.

The papillae were, in general, shorter and broader on isolated conceptuses when these were compared with profiles within the glandular lumina. This suggested a tight fit, with some constraint on the papillae by the gland. The ease with which intact conceptuses can be flushed out of the uterus up to day 16 does not indicate a very firm adhesion between gland and papilla. However, uterine glandular lumina containing broken-off papillae, and the finding of fragments of papillary apical membrane adherent to uterine epithelial microvilli, suggests a significant degree of interaction between the maternal and fetal membranes, although it is impossible to estimate the contribution of fixation to these adhesions.

It seems a reasonable assumption that papillae observed on isolated conceptuses on days 13–15 were formed inside a glandular lumen and were not just random outgrowths of the trophectoderm. It is clear that the trophectoderm plus endoderm can grow down into folds which are narrower than a papilla, and that, therefore, the lack of endoderm or trophectoderm basement membrane in a papilla indicates that it is a specialised structure which is formed in a restricted region and for a definite purpose. At the earliest stage at which trophectoderm was observed apposed to the uterine epithelium, at day 16, all the papillae on the sections were associated with gland openings.

There are at least three obvious possible functions for the papillae: to serve as pegs for anchorage, or to serve for output or uptake of nutrient or messenger

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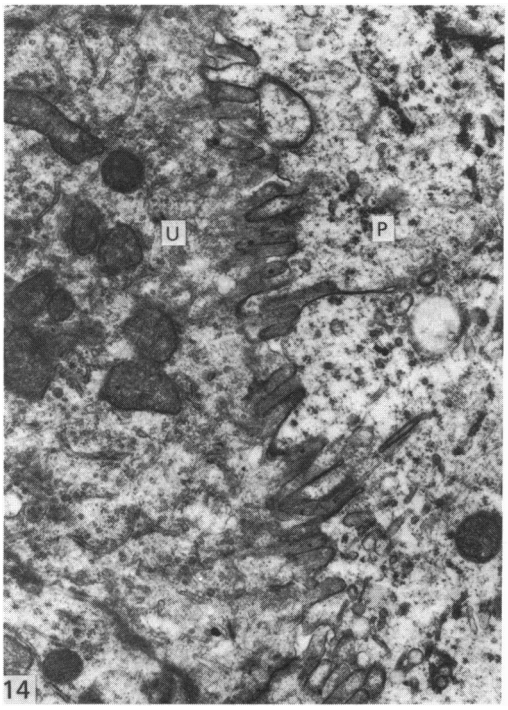
Fig. 11. 16 days old sheep conceptus fixed *in utero*. An electron micrograph of the papilla shown in Fig. 6 showing the similarity of the cells in the papilla (P) to the cells of the trophectoderm (T). One non-granulated binucleate cell (B) can be seen halfway down. The trophoblast endoderm (arrowheads) can be seen to run across the base of the trophectoderm and does not penetrate into the papilla. × 800.

Fig. 12. 17 days old sheep conceptus fixed *in utero*. Part of a transverse section through a papilla (P) deep in a uterine gland (G). The papillary cells characteristically are linked by tight junctions (arrowheads), contain lipid (L), crystal-containing vacuoles (arrow) and a large nucleus (N). × 3000.

Fig. 13. 17 days old sheep conceptus fixed *in utero*. Electron micrograph showing a dense mass of (degenerated?) material (arrow) including crystals and lipid droplets at the tip of a papilla. This may represent the start of the breakdown of the papilla. Gland epithelium (G). × 660.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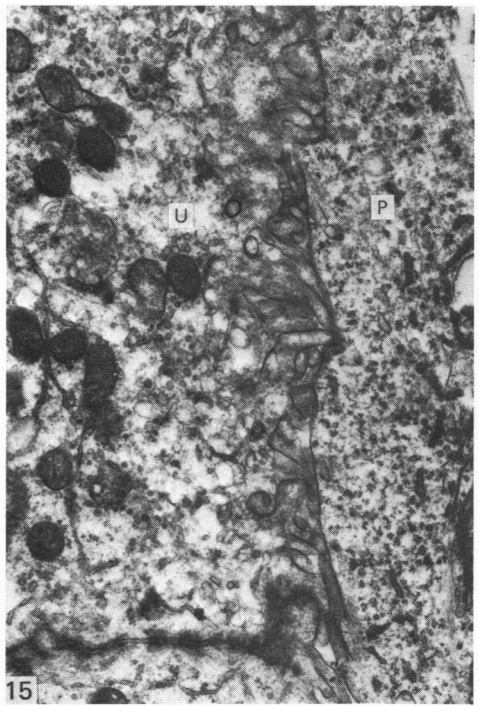
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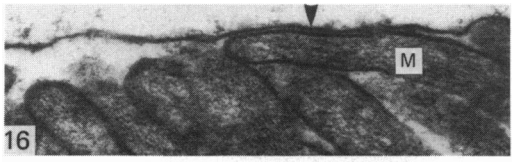
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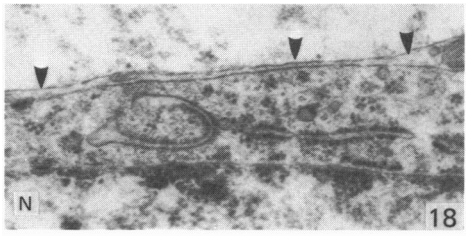
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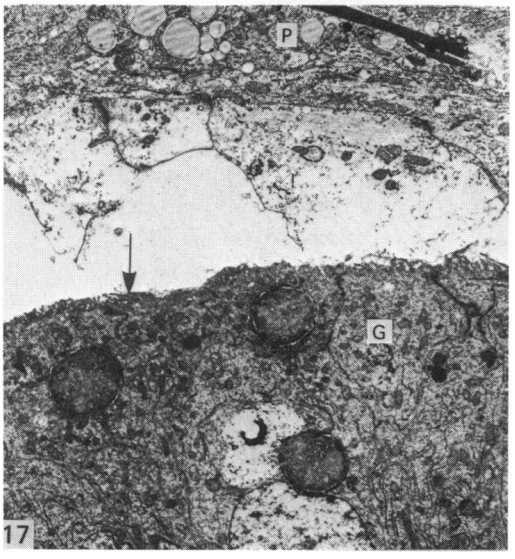
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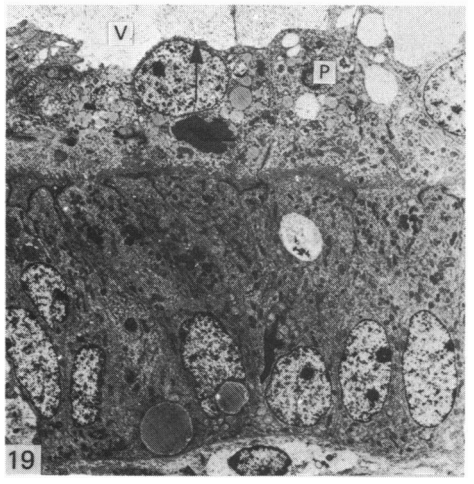
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molecules. The lack of any significant difference in the ultrastructure of individual cells of the papillae and those of the rest of the trophectoderm would indicate an equivalence of synthetic and uptake capacity. As a group, the cells of the papillae are no nearer the maternal capillaries than are the other trophectodermal cells, and transport of materials down a solid rod of cells would presumably be less efficient than that occurring through the monolayer of chorion cells confronting the surface of the uterine epithelium. Transport of nutrients to the embryonic mass, for example, would not be enhanced by the presence of papillae. No evidence was found that the papillae were clustered preferentially immediately around the site of the embryo in the centre of the conceptus. Instead, they were evenly scattered in that general region where implantation starts in the sheep (Boshier, 1969; Lawn, Chiquoine & Amoroso, 1969).

It is possible that the close inter-relationship between gland opening and papilla may play a role in the signalling system from the uterus and/or conceptus for maintenance of the corpus luteum required around day 12–13. However, since the interaction with the gland epithelium seemed to be the same as elsewhere, such a specific role apears unlikely.

The papillae are well developed two or three days before the first reported time of the cellular changes which produce binucleate cells in the chorion and multinucleate or giant cells in the uterine epithelium (Boshier, 1969). These changes were previously the best visible indicators that implantation had started.

The conceptus elongates enormously between days 12 and 16 and some means of immobilising the rapidly developing embryonic disc region as close to its nutrient source as possible would seem to be advantageous. Also, the fact that the papillae have not been observed at or after 20 days of gestation suggests that their most probable function would be localisation and anchorage during the earliest stages of implantation. This would bring the chorion into the very close relationship with the uterine epithelium that is required for the subsequent cellular changes leading to formation of the definitive placenta.

SUMMARY

A study of 13–18 days old sheep conceptuses has consistently demonstrated the presence of multicellular protrusions (papillae) from the trophectoderm surface. These papillae were shown to be restricted to the embryonic region of conceptuses flushed out of the uterus. After perfusion fixation of the uterus on day 16 of preg-

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Figs. 14–15. 16 days old sheep conceptus fixed *in utero*. Electron micrographs showing apposition between the apical microvilli of a papilla (P) and the uterine epithelium (U). These represent the two extremes of the range of microvillar interdigitation found, Fig. 15 being the more characteristic. × 5000.

Figs. 16–17. 16 days old sheep conceptus fixed *in utero*. Electron micrographs showing cells of the papilla (P) pulled away from the uterine gland epithelium (G). In Fig. 16, the region indicated by the arrow in Fig. 17 is enlarged to show how the apical plasmalemma (arrowhead) of the papillary cells remains adherent to the microvilli (M) of the uterine epithelium. Fig. 16 × 46000; Fig. 17 × 2000.

Figs. 18–19. 16 days old sheep conceptus fixed *in utero*. Electron micrographs showing part of a 'vacuolated' (V) papilla (P). In Fig. 18, the region at the edge of the 'vacuole' indicated by the arrow in Fig. 19 is shown at higher magnification. Nucleus (N). Note the continuous membrane (arrowheads) which borders the papillary cell membrane, suggesting that the 'vacuole' is formed by cell rupture. Fig. 18 × 26000; Fig. 19 × 1000.

nancy, the conceptus papillae can be observed penetrating well down into the lumina of the uterine glands. The papillae have not been observed at or after day 20. It is suggested that the papillae may play an important but transient role in anchoring the embryonic region of the conceptus against the uterine epithelium to allow the initiation of the cellular changes characteristic of implantation.

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