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Cellular Basis of Interaction Between Trophoblast and Uterus at Implantation

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INTRODUCTION

Implantation marks a transition stage in the progress of pregnancy during which the blastocyst assumes a fixed position and begins an altered physiological relationship with the uterus, but it is not a single event, nor can it be established as occurring at a single instant in time. It is nevertheless possible to restrict our view of implantation and to discern some general features by considering that, despite variations in levels and ratios of estrogens and progesterone, structure of the endometrium, and size of the blastocyst, implantation in mammals always involves a direct interaction of the trophoblast of the blastocyst with the luminal epithelium of the uterus.

Because of recent progress in the study of cellular interactions during differentiation and interesting suggestions concerning physiological mechanisms involved in the initiation of implantation, it seems particularly appropriate to take an overall view of the morphological context of the events in the process of implantation, and to suggest in which areas these events have implications concerning the cellular mechanisms that may be involved in this crucial event in development. In addition to emphasizing general biological features, consideration of the specific cellular events of implantation in their sequence helps to avoid the siren song simplicity of single mechanisms, substances or stimuli as the causative agent of implantation.

ADHESION OF TROPHOBLAST AND UTERINE EPITHELIUM

In discussing implantation, it is useful to separate the early events into a series of processes. The first associations between blastocyst and uterus that establish the definitive position of blastocyst in relationship to the uterus are frequently referred to as attachment. This term, however, seems a bit too finite, with connotations of instant and permanent fastening. More realistically, apposition of trophoblast to uterus must be brought about before any adhesion can develop, and adhesion *per se* is probably progressive rather than instantaneous. It is therefore convenient to divide this attachment stage of implantation into an initial appositional stage and a subsequent adhesion stage that may or may not be initiated at the beginning of apposition. Following the establishment of position of the blastocyst, the subsequent stage of epithelial penetration can progress in an orderly fashion, restricted to appropriate regions of trophoblast-uterine association.

Since attainment of apposition is a divergent phenomenon involving a variety of physiological mechanisms, some of which are not directly related to trophoblast-uterine cullular interactions, this stage will not be considered. Recent reviews including some discussion of this area are those of Enders (1972) and Psychoyos (1973a).

Morphological Evidence of Adhesion

One of the most interesting aspects of the morphological studies of the last decade has been the finding that in all species studied, no matter what the means of accomplishing apposition of blastocyst to uterus, there is direct adhesion of trophoblast to uterine luminal epithelium prior to penetration of this epithelium (Enders, 1972). Even species which do not have epithelial penetration, for example those that form an epitheliochorial

placenta, have stages in which the cellular association of trophoblast with uterus is sufficient that the developing conceptus cannot be removed without damage (Björkman, 1973).

Adhesion at implantation refers to those forces resisting displacement of the blastocyst from the uterine epithelium at their sites of contact. There are a number of cytological criteria indicating different degrees of adhesion. Initial studies of early implantation emphasized the interdigitation of microvilli of the two epithelia in a number of species (Reinius, 1967; Enders and Schlafke, 1967; Tachi, Tachi and Lindner, 1970). The change in uterine state which results in interdigitation can be seen not only in increased intimacy of association between blastocyst and uterus but also in the interdigitation of microvilli of the apposed surfaces of the uterine luminal epithelium. This 'attachment reaction', during which the uterine surfaces are approximated, obliterating the uterine lumen, has been shown to occur in a number of species (rat: Ljungkvist, 1972; mouse, hamster and guinea pig: Hedlund, Nilsson, Reinius and Aman, 1972) but not in others (rabbit and mink: Hedlund *et al.*, 1972). Initial stages of the 'attachment reaction' are apparently rather labile. For example, in the rat and mouse, gentle perfusion of fluid into the uterine lumen permits separation not only of microvilli of the two adjacent walls of the uterine lumen but also the microvilli of trophoblast from those of the adjacent uterine epithelium.

Subsequent to interdigitation of microvilli, there are two types of evidence for increasing adhesion of trophoblast to uterus. One type consists of distortion of one or the other surface, either normally, such as in the ferret where tongues of uterine tissue are drawn up into the trophoblast (Fig. 12), or in partial displacement during processing where strands of cytoplasm are drawn out between the two surfaces thereby illustrating regions of intimate membrane association.

The second type of evidence is that of formation of junctional complexes. These consist of typical junctional complexes found at lateral borders of epithelial cells which appear to be shared with the invading trophoblast, and of less clearly defined junctions on the apical surfaces of epithelial cells.

There is a progressive establishment of regions of very close (less than 200 Å) membrane association between trophoblast and apical cell surfaces of the uterine epithelium (Figs. 1, 8). In such regions of close association uterine microvilli adjacent to trophoblast become irregular in shape, and there are extensive areas in which cell membranes are parallel and closely apposed (Enders and Schlafke, 1967; Potts, 1968; Tachi *et al.*, 1970; Fig. 1). Although specializations within such junctional complexes (septate desmosomes, etc.) have been described, the more common observation is of little apparent specialization other than prominent ectoplasmic regions in either or both cells, often containing fine filaments.

Recently a great deal of information has been added to our understanding of junctions by freeze-cleave techniques (McNutt and Weinstein, 1973), by the use of tracers of extracellular space (Friend and Gilula, 1972), and by studies of electrical coupling between cells (Bennett, 1973). Because so much additional information about junctions can be achieved by these special methods, junctions which have been studied only by transmission electron microscopy cannot be considered to have been completely characterized. At the present time, the description of special areas of association between trophoblast and uterus is useful in showing that there is intimacy of association, and that specialization for adhesion is present in such regions. However, application of more recently developed methods for study of junctional complexes would add greatly to our understanding of the nature of these associations.

Theory of Cellular Adhesion

Since adhesion is one of the earliest significant cellular events in implantation, the possible mechanisms of this adhesion should be considered. Although it would be both inap-

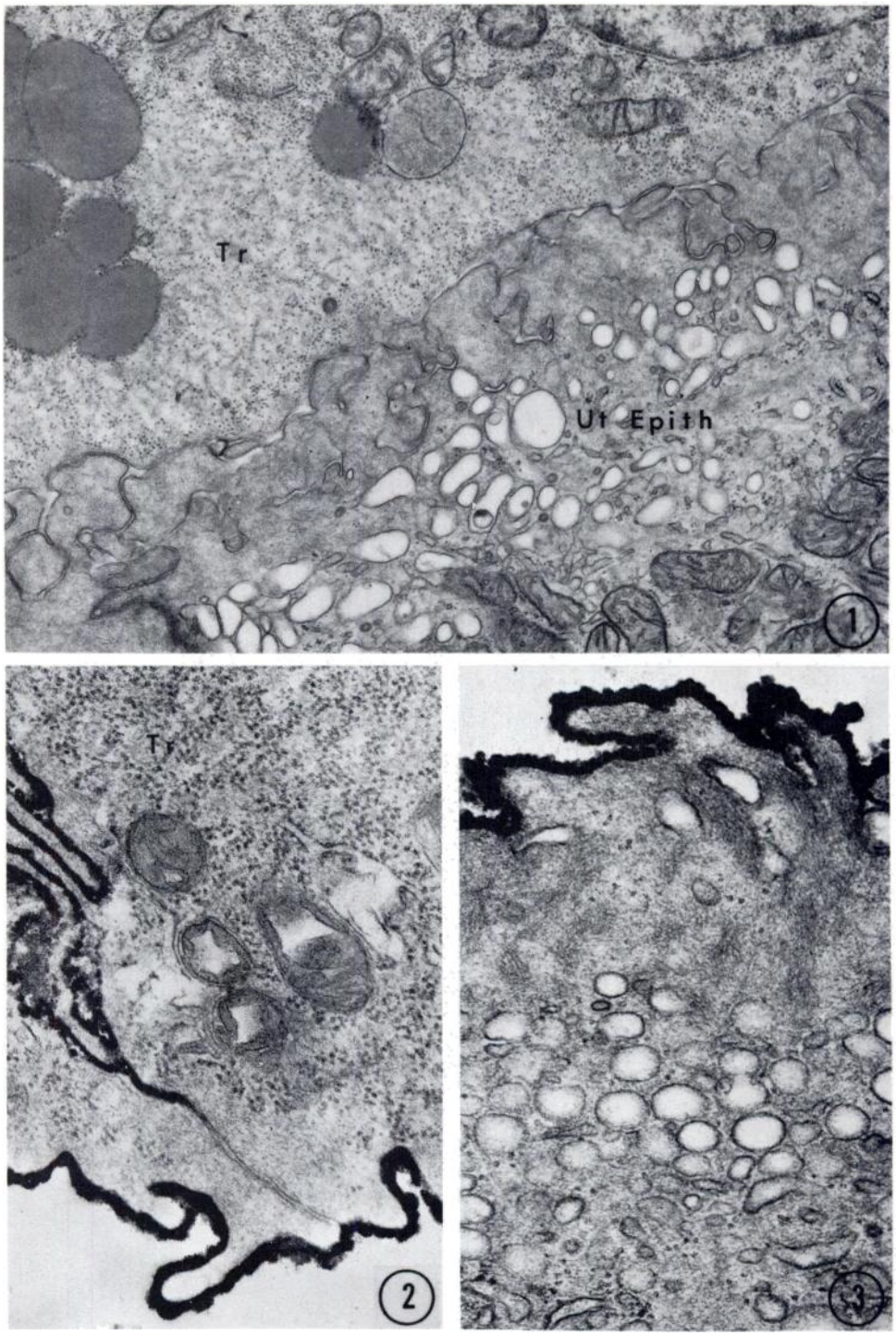


FIG. 1. A trophoblast cell (Tr) of a rat blastocyst is closely adherent to the apical surface of uterine epithelial cells (Ut Epith) in this electron micrograph of an implantation site on day 6 of pregnancy. Uterine microvilli are blunt and irregular and are composed of ectoplasm containing microfilaments. A zone of clear vesicles is seen beneath the ectoplasmic layer of the uterine cell. ×21,600.

FIG. 2. The surface of a mouse blastocyst which had been treated with the concanavalin A-peroxidase method to demonstrate sugars (especially mannose) on the cell surface. The marker has reached the intercellular space on the left through damaged cells outside the field of the micrograph. Day 5, 9 a.m. ×50,400.

FIG. 3. Surface of mouse uterine epithelium similarly treated with concanavalin A. Like the blastocyst, the uterine surface binds this phytohemagglutinin. Day 7, delayed implantation. ×50,400.

propriate and presumptuous to attempt to review such an extensive and controversial field as cellular adhesion, it is necessary to present a brief summary of information concerning our current knowledge in this interesting area before considering the special case of adhesion in implantation. Relationships between cell adhesion and locomotion during trophoblast invasion will be considered later.

It has been established that all cells have surface glycoproteins as a part of their limiting membrane (Rambourg and Leblond, 1967; Cook and Stoddard, 1973). Changes in surface components of cells in different functional states have been seen during mitosis (Warren and Glick, 1968), in active phagocytosis (Allison, 1973; Oliver, Ukena and Berlin, 1974), in cell transformation (Burger and Noonan, 1970), in embryonic development (Moscona, 1971), and in cell adhesion in vitro (Bosmann, 1972, 1974; Bosmann and Winston, 1970). Although glycolipids and pure proteins are found at cell surfaces, attention has been focused principally on the glycoproteins (Cook and Stoddart, 1973). The most extensively studied model for these molecules is that of erythrocyte ghosts (Marchesi, et al., 1972; Steck, 1974). In the erythrocyte membrane the glycoprotein consists of a polypeptide chain anchored within the cell membrane in association with intramembrane protein, and a portion projecting above the surface of the membrane containing a number of oligosaccharides. In addition, in a number of developing systems glycosyltransferases have been shown in association with the free surface of the cells.

The specific molecules involved in adhesion would be expected to be those sufficiently terminal to be stereologically available. Change from a non-adhesive to adhesive state implies a shift in number, distribution or position of some of the molecules of the surface coat. Progressive increase in adhesion could involve synthesis, that is, addition of molecules not previously present on the cell surfaces. Alternatively, the specific reactive molecules could be made available by lysis of portions of the surface molecules, thus exposing groups not previously available. An additional possibility is a change of configuration or the linear translocation of cell surface molecules to form an aggregate "patch" participating in adhesion.

The hypothesis that glycosyltransferases could act as linking molecules is useful both as a possible mechanism of adhesion, and as an illustration of the requirement for establishing a specific intermembrane association (Roseman, 1970). The glycosyltransferases are enzymes that catalyse the addition of sugars to oligosaccharides. They are specific not only for the sugar added but also for the glycoprotein receptor to which it is added, and they need the sugar in a specific nucleotide form as a substrate. They thus have a high degree of specificity, and would serve as a link by bridging from the surface in which they are situated to an available sugar on the adjacent cell surface (Roth, McGuire and Roseman, 1971; Roth and White, 1972). Weiser (1973) examining glycosyltransferase activity in intestinal cells, pointed out that the less differentiated crypt cells have glycosyltransferase activity and receptors which are not found on the villi. Jamieson, Urban and Barber (1971) and Hay (1973) illustrated how these mechanisms, including glycosyltransferase activity, may apply in adhesion to substrates.

At this stage in the study of adhesion, there is clearly no consensus concerning the molecular processes involved. However, the cell surface materials, especially glycoproteins, play a prominent role in most of the current suggested mechanisms.

Cell Surface Material at Implantation

Initial investigations into the cell coats of mammalian embryos were begun by Cooper and Bedford (1971), who reported an increase in the density of colloidal iron hydroxide staining material on the rabbit egg cell membrane after fertilization. Yanagimachi, Nicolson, Noda and Fujimoto (1973) examined cell surfaces at the time of interaction of hamster ova and sperm at fertilization, using the colloidal iron hydroxide method. They dem-

onstrated acidic anionic residues at the surface of the ovum, but showed no alteration of location or density after fertilization.

Investigations of the surfaces of cells which will participate in implantation is just beginning to receive attention. Pinsker and Mintz (1973) analyzed cell membrane components of mouse two- and four-cell stages and morula-early blastocyst stages using incorporation of tritiated glucosamine. After incubation with the tritiated sugar, the embryos were exposed to trypsin to remove cell surface components. The resultant material was digested in pronase for analysis. Their results indicate that there is a greater amount of cell surface material available to trypsin digestion in the morula-blastocyst stages, and that the label is incorporated into higher molecular weight fragments. This study used tritiated glucosamine, which would be expected to be converted into glucose as well as other amino sugars (Kornfeld and Gregory, 1968). Amino sugars are usually associated with the inner residues of the carbohydrates adjacent to the protein backbone. It would be of interest to examine some of the sugars which are normally located in a terminal position, for example sialic acid or fucose and their relationship to trophoblast-uterine adhesion.

Holmes and Dickson (1973) used Prussian blue staining of whole mouse blastocysts to determine the concentration of colloidal iron stain on the negative surface sites of the blastocyst. They reported that the staining achieved by this somewhat indirect method appeared only after blastocysts had been exposed to estrogen, and that blastocysts during delayed implantation or after incubation with neuraminidase were unstained.

Conversely, Nilsson, Lindqvist and Ronquist (1973) reported that mouse blastocyts after estrogen exposure appeared to have less affinity for colloidal iron staining, as observed with electron microscopy, than those exposed to progesterone alone. They concluded that there is a diminution of the net negative charge on the activated blastocysts.

Using a variety of stains which indicate at the electron microscope level characteristics of cell surface glycoproteins, Enders and Schlafke (1974) examined mouse blastocysts and uterus on day 4 (preimplantation stages with zona-encased blastocyst), day 5 (implantation stages, blastocysts without zonas) and on day 7 of lactationally delayed implantation. Binding of concanavalin A and staining with both ruthenium red and colloidal thorium indicated the presence of acidic glycoproteins on blastocysts and uterus at all stages examined (Figs. 2, 3). Although some minor differences in stain thickness were seen, the problems associated with the nature of the methods and irregularity of the cell surfaces prevent accurate quantitation.

It has also been suggested that alteration of the surface charges might cause adhesion of trophoblast and uterus (Clemetson, Kim, Mallikarjuneswara and Wilds, 1972). While charge differences may be involved in some manner, Levin (1973) has pointed out that most studies thus far have confused a negative transluminal membrane potential difference with a net negative charge on the surface *per se*. Since most cell surfaces carry a negative surface charge, it seems unlikely that adhesion is the result of electrostatic surface charges on the two cell types.

In several species (ferret and mink), the "glycocalyx" on the uterine surface is extremely thick and can be visualized in transmission electron microscopy without histochemical methods. In the ferret at the time of implantation it is clear that this coat is removed as apposition of cell membranes occurs (Enders and Schlafke, 1972).

Since there is close apposition of cell membranes during the adhesion stage of implantation in all species examined so far, reduction of cell surface materials may well be a general phenomenon. Although lysis of the thicker uterine cell coat is the most obvious possibility, rearrangement and molecular intermingling could account for some of the apparent reduction in thickness.

If there is a modification of uterine cell surface at the specific localized site of implantation, it is important to examine the region which is in contact with the blastocyst rather

than extrapolating from observations of the entire uterine surface. In approaching this problem, we have recently been attempting to develop a method whereby the surfaces of the blastocyst *in situ* and the uterine surface of the implantation chamber adjacent to the blastocyst can be observed. Implantation sites on day 6 in the rat are carefully split in half along the mesometrial-antimesometrial plane. The surfaces exposed reveal the uterine implantation chamber and the surface of the blastocyst on one wall (Figs. 4, 5), and the surface of the implantation chamber bearing the imprint of the blastocyst on the contralateral wall. Scanning electron microscopy of these surfaces has demonstrated the progressive flattening of microvilli of the uterus adjacent to the blastocyst (Fig. 6).

In addition, the method provides an illustration of the progressive increase in adhesion of blastocyst to uterus. In early developmental stages (late day 5, early day 6 in the rat), the blastocysts tend to be dislodged from the chamber during splitting. With progressive development as seen by increase in depth of the implantation chamber, the blastocysts adhere to one side of the chamber with the imprint from which it was dislodged being visible on the contralateral surface. With further development, one or more blastocysts may be split during the separation of the two halves. By late day 6 or early day 7, all successfully split chambers pass through the blastocyst, and it is no longer possible to obtain an imprint on uterine epithelium that had been adjacent to trophoblast.

Future Studies

The numbers of procedures available for further studies of surfaces of blastocyst and uterus at implantation are as numerous as the approaches to the study of cell surfaces in general. The more obvious methods, in addition to those already presented, include introduction of tritiated sugars *in vivo* for conversion and utilization in neoformation of cell surface materials. Conversely, the appropriate tritiated sugar nucleotides may be introduced directly into the uterus *in vivo* or *in vitro* to determine the presence or absence of specific surface glycosyltransferases at the time of implantation. Comparative exposure to phytohemagglutinins with affinities for different specific sugars could be used in connection with their competitive sugars to demonstrate relative availability of the specific sugars in the coats. Differential enzyme digestion, both by individual glycosylases and by endopeptidases could be used not only to analyze the exposed groups within the cell surface materials but also to determine whether alteration of these materials changes adhesiveness.

For study of change of adhesiveness, a model system would be necessary. Exposure to appropriately treated cell suspensions or smaller particles might constitute a means by which some standard measurement of blastocyst surface adhesion could be achieved. Alternatively, the *in vitro* outgrowth of trophoblast (Gwatkin, 1969; Jenkinson and Wilson, 1973) could be used both to ascertain cell surface differences between outgrowing and non-outgrowing trophoblast cells and as a basis for study of experimental alteration of trophoblast adhesiveness.

Although this area is clearly open to imaginative investigation, it must be kept in mind that trophoblast adheres to a specific area of the uterus, and that this is an *interaction* involving both participants, not a single isolated event.

PENETRATION OF UTERINE EPITHELIUM BY TROPHOBLAST

Examination of trophoblast of different species at the stage of penetration into the endometrium gives the impression that this tissue is somehow more "active" or more invasive in some species than in others. During implantation in the mouse and rat, for instance, trophoblast may remain at the surface of the epithelium for a day, and subsequently pause at the basal lamina of this epithelium for yet another day. In contrast, epithelial penetration in the guinea pig is so transient that it is a difficult stage to collect. This subjective impression of trophoblast

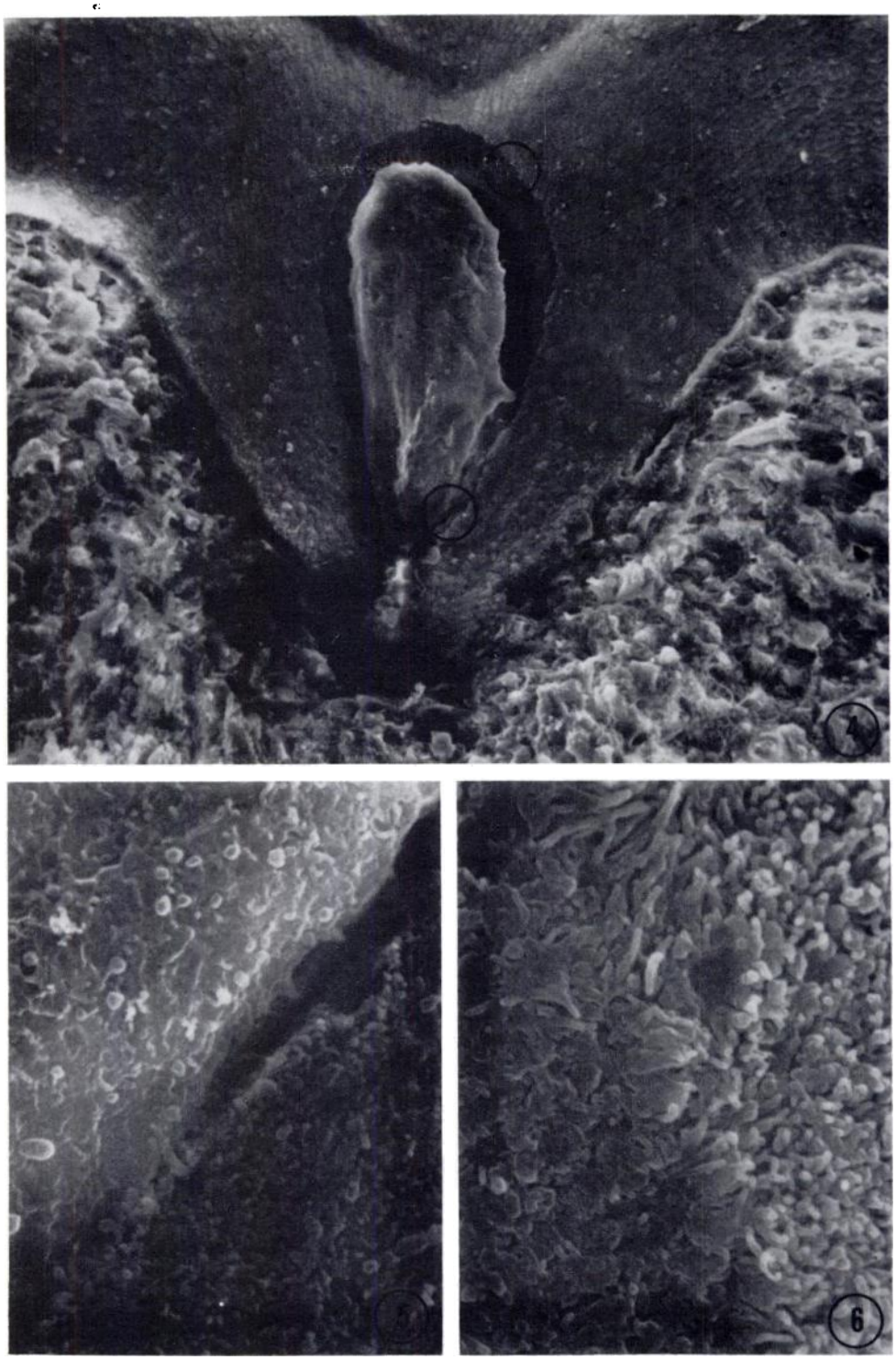


FIG. 4. Scanning electron micrograph of a rat blastocyst on the surface of the implantation chamber of the uterus. The blastocyst has pulled away slightly from the uterine surface, showing the imprint of the area previously occupied by the blastocyst. Day 6, ×550. (Reprinted with permission from Enders, A., 1975, "The implantation chamber, blastocyst, and blastocyst imprint of the rat: a scanning electron microscope study." *Anat. Rec.*, in press).

FIG. 5. Scanning electron micrograph of an area similar to that in the lower circle of Figure 4. The surface of the trophoblast (upper left) is relatively smooth, showing principally small ridged protrusions. The surface of the uterus (lower right) is covered with microvilli. Day 6. ×5,000.

FIG. 6. An area similar to that in the upper circle of Figure 4. The surface of the uterus which was adjacent to trophoblast was blunt with irregular microvilli (lower left), while that which was adjacent to the contralateral uterine epithelium has thinner, more elongate microvilli (upper right). Day 6. ×8,000.

activity could be made more objective if the uteri of different species of mammals were more similar in both structure and in their response to trophoblast. In such a situation, rates of penetration of trophoblast could be established and considered active or passive accordingly. However, the structural organization of the uterus at the site of implantation, whether the epithelium has a tendency to dislodge, whether there is an epithelial proliferation or stromal decidual reaction, and how early these occur all may influence the apparent invasiveness of the trophoblast.

It is possible, however, to classify the process with regard to the nature of the involvement of the uterine epithelium (Fig. 15). The types of interrelationship observed in species studied to date appear to fit into three categories:

(a) **intrusive implantation**, in which trophoblast penetrates between apparently healthy uterine luminal epithelial cells to the basal lamina of the uterine epithelium, then extends beneath this;

(b) **displacement implantation**, in which the uterine epithelium is readily released from the underlying basal lamina, facilitating spread of trophoblast through and beneath the epithelium; and

(c) **fusion implantation**, in which the first penetration of epithelium by trophoblast is accomplished by fusion of an area of syncytial trophoblast to individual uterine luminal cells.

Intrusive Implantation

Judging from the evidence from light microscopy and from later stages in implantation, intrusion of trophoblast between uterine luminal epithelial cells would appear to be a likely method of penetration of this layer in a large number of species (Enders, 1972). The general observation of apposition of trophoblast to healthy epithelium, and penetration without extensive destruction or alteration of adjacent cells of this epithelium are the types of evidence that lead us to suspect trophoblast intrusion. Although intrusion could theoretically occur at a single spot on the endometrial surface, in most cases several areas of penetration occur, depending in part on the size of the blastocyst and the amount of blastocyst surface participating in the invasion process.

The ferret is a useful example of intrusive implantation. It can be readily studied since the blastocyst is large, and penetration is accomplished at a number of places over much of the antimesometrial surface of the implantation chamber (Enders and Schlafke, 1972).

The first areas of adhesion and penetration are formed by syncytial plaques that develop on the lateral antimesometrial walls adjacent to the antimesometrially-situated embryonic cell mass during day 12 post coitus. Unusual ectoplasmic pads of syncytial trophoblast adhere to the uterus and often indent this epithelium (Figs. 7–9). Subsequently thin flanges or folds of syncytium apparently penetrate between apical ends of uterine epithelial cells (Figs. 8, 10). These processes later extend to the basal lamina of the epithelium. The trophoblast processes share the uterine apical junctional complexes. The sequence of components of the junction is the same as in junctions between uterine epithelial cells adjacent to the implantation sites. As yet the mechanism of breaching of the uterine apical junctional complexes is unknown. Shared desmosomes are also common between intruding trophoblast and the lateral cell margins of uterine epithelium (Fig. 8).

At the time of penetration, both the initial ectoplasmic pads that adhere to the uterus and the tongues of syncytium that intrude between uterine cells contain numerous fine filaments and occasional microtubules. As the area of attachment to the uterus increases, the region of adhesion of syncytium to uterus becomes more extensive, and the apical ends of some of the uterine cells are distorted. Where the ectoplasmic pads are closely adherent to the uterine cells, there are filamentous regions within the subjacent regions of these latter cells as well as in the trophoblast. In addition, extracellular homogeneous material intervenes irregularly between trophoblast and uterine epithelium. This material is

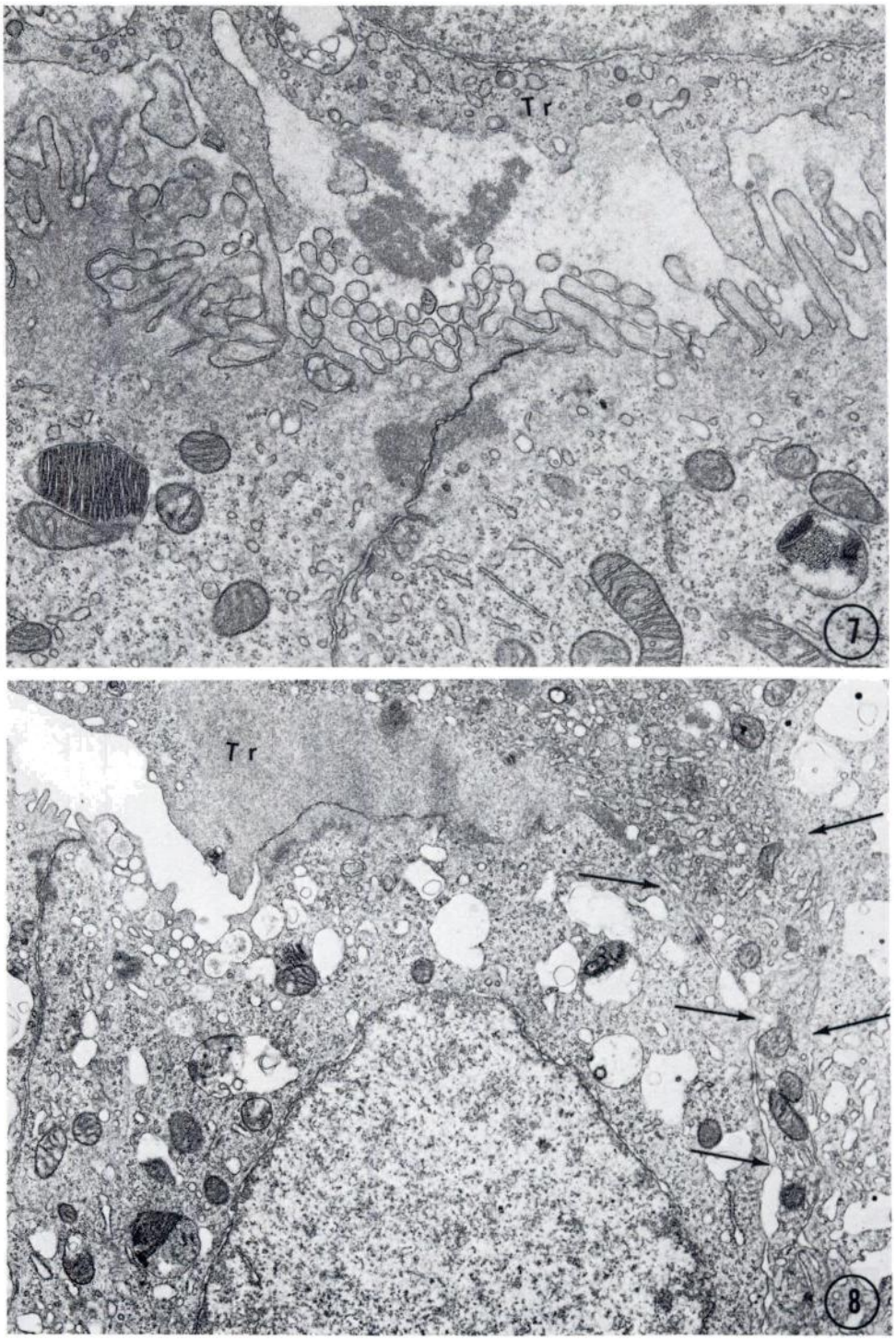
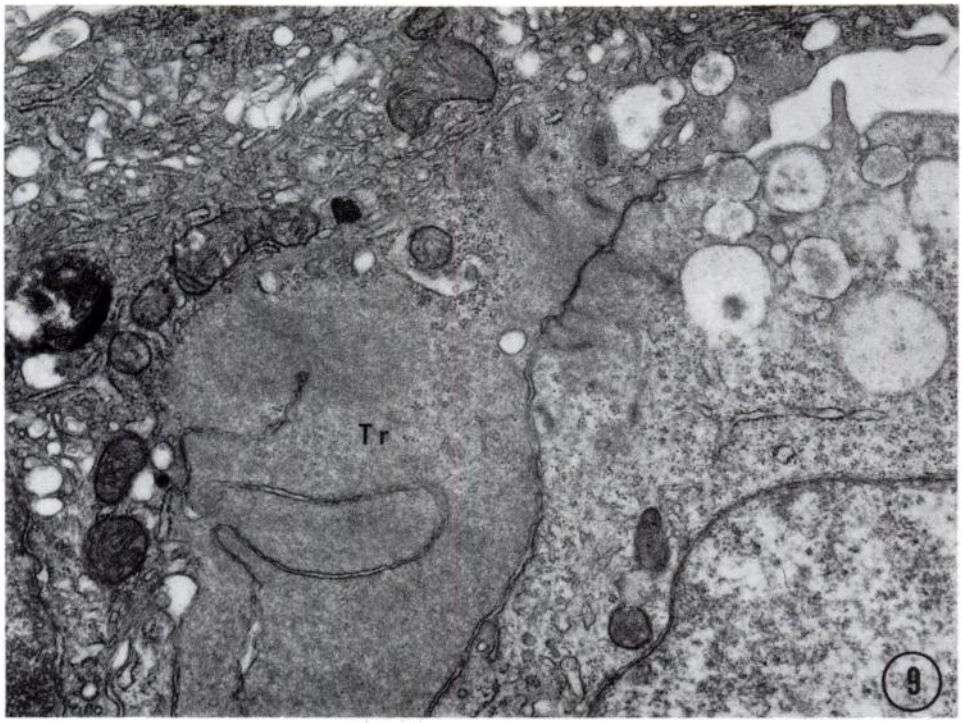


FIG. 7. In this electron micrograph of a ferret implantation site, occasional uterine microvilli are contacted by blunt ectoplasmic projections of the trophoblast surface (Tr). Remnants of the zona pellucida are seen in the center of the space between the two epithelia. Two membranous granules are seen in the uterine cells. 12 days p.c. ×23,000.

FIG. 8. An area where trophoblast (Tr) of the ferret blastocyst adheres to the apical surface of uterine epithelium and intrudes between uterine cells (arrows). An extensive ectoplasmic region of trophoblast is apparently closely adherent to uterus. The large vacuoles in the apical region of uterine cells adjacent to the trophoblast are not a consistent finding. 12 days p.c. ×13,000.



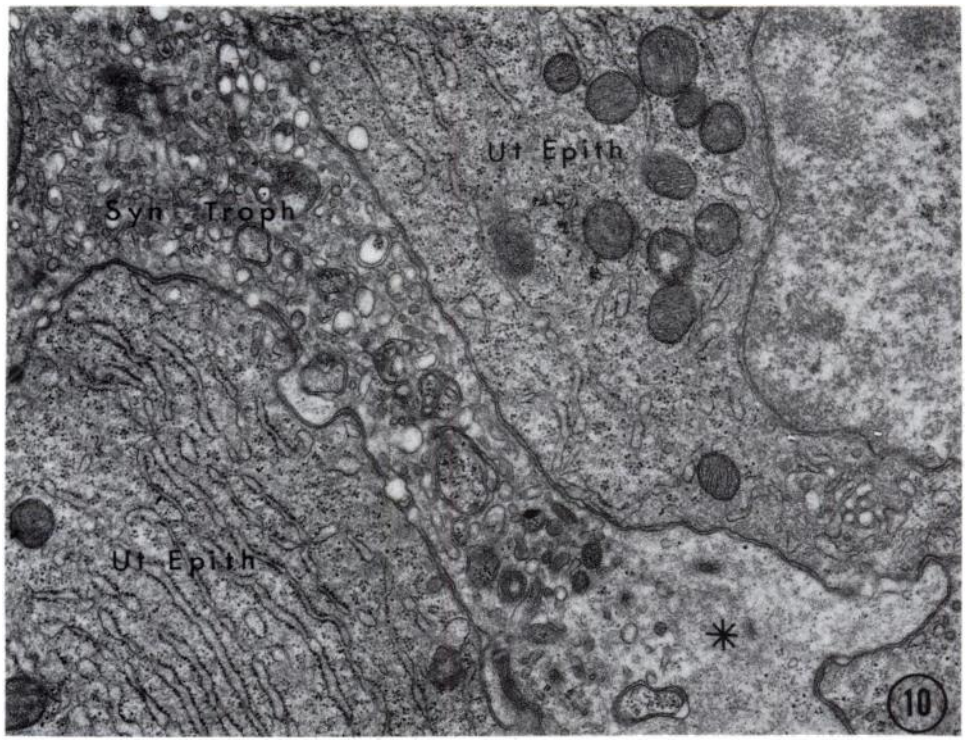


FIG. 9. Ferret implantation site. Trophoblast (Tr) contains an ectoplasmic pad which is adherent to the lateral cell membrane of a uterine epithelial cell (right). Note the infolding of the ectoplasmic area. 12 days p.c. ×21,200.

FIG. 10. Area of trophoblast intruding between epithelial cells in a ferret implantation site. The trophoblast process (Syn Troph) contains both an ectoplasmic area (asterisk) and regions of vesicles and granules. The uterine epithelial cells (Ut Epith) appear healthy, with cell membranes closely apposed to trophoblast. 12 days p.c. ×24,000. (Reprinted from *Amer. J. Anat.*, **125**, 1–30, 1969).

similar in density to that in granules in adjacent trophoblast, and appears to be secreted by this tissue.

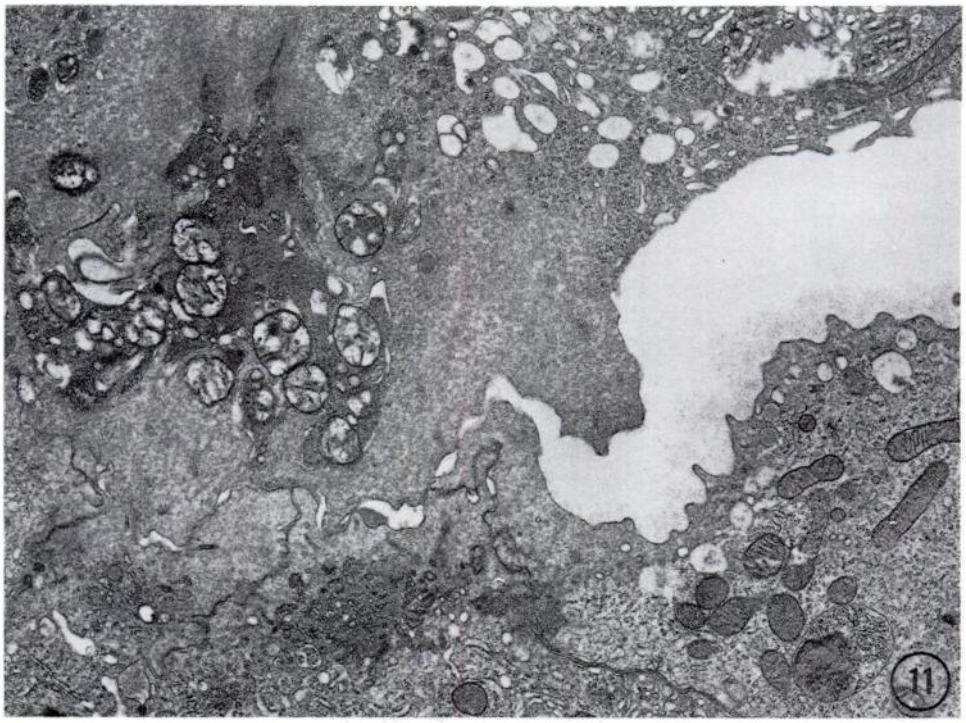
The larger projections of trophoblast intruding between epithelial cells contain small vesicles, agranular endoplasmic reticulum, and a few mitochondria, and are consequently not strictly ectoplasmic. The ectoplasmic zones appear to be associated with adhesion, and not necessarily with the projecting tongues of cytoplasm that intrude between the epithelial cells.

Subsequent invasion of trophoblast into maternal stroma continues rapidly, with syncytial trophoblast penetrating deeper into the endometrium to surround blood vessels and glands within 24 hours (Enders and Schlafke, 1972; Gulamhusein and Beck, 1973).

An interesting example of intrusion by cellular trophoblast has recently been documented by Allen, Hamilton and Moor (1973) in the mare. In this instance, the trophoblast penetrates from its position in the chorionic girdle into the endometrium, not as a mechanism of attachment of the blastocyst at implantation but in the process of forming endometrial cups responsible for secretion of pregnant mares serum gonadotropin. Ectoplasmic areas containing filamentous material develop in the cellular trophoblast and adhere to the apices of uterine epithelial cells. Subsequently projections from the ectoplasmic portions of the trophoblast cells indent the uterine epithelial cells. With continued development and enlargement of ectoplasmic processes, the uterine epithelial cells are engulfed and removed by phagocytosis. It is not clear whether the trophoblast at any point intrudes between the individual uterine cells by breaching junctional complexes, or to what extent the cells are fragmented prior to phagocytosis, but it does seem clear that the initial penetrations are into the substance of the cell rather than between cells. The trophoblast cells that have removed the uterine epithelium then migrate into the stroma, where they take up residence as endocrine cells.

Other species in which evidence currently appears to point to intrusion implantation include the guinea pig. The guinea pig implantation cone, which consists of a region of syncytial trophoblast at the abembryonic end of the blastocyst, can be seen to penetrate the zona pellucida via ectoplasmic trophoblastic processes (Blandau, 1949; Enders and Schlafke, 1965, 1969; Parr, 1973). These processes apparently adhere to uterine epithelium and rapidly penetrate through this epithelium. There are few uterine epithelial cells displaced during these stages, and little phagocytosis of uterine epithelium is seen (Blandau, 1961; Deanesly, 1971). Due to the rapidity of this stage, the few blastocysts, and the large size of the guinea pig uterus, it has been difficult to obtain adequate material for cytological study. Electron micrographs of this stage reveal numerous irregular ectoplasmic processes projecting from the syncytial implantation cone. In one implantation site prepared for electron microscopy, trophoblast is tightly adherent to uterine epithelium, causing some distortion of the underlying uterine cells (Enders and Schlafke, 1969). However the quality of the material and the fact that it is adjacent to the confluence of glandular epithelium with luminal epithelium prevents a definitive statement concerning method of penetration.

Early stages in implantation in primates have yet to be clarified by cytological examination. The earliest human implantation site which has been described by electron microscopy can be estimated to be 11 days post coitus, is well beneath the epithelial surface, and is developed to the lacunar stage (Knoth and Larsen, 1972). Electron microscopic examination of a single early stage in the rhesus monkey provided somewhat confusing results. In this specimen cellular trophoblast is intermingled with cells of the uterine epithelial plaque (Reinius, Fritz and Knobil, 1973). Syncytial trophoblast was not seen, and it is difficult to resolve the border of the advancing trophoblast in the micrographs. Heuser and Streeter (1941) illustrate two stages in implantation in the rhesus which are apparently slightly earlier than that described by



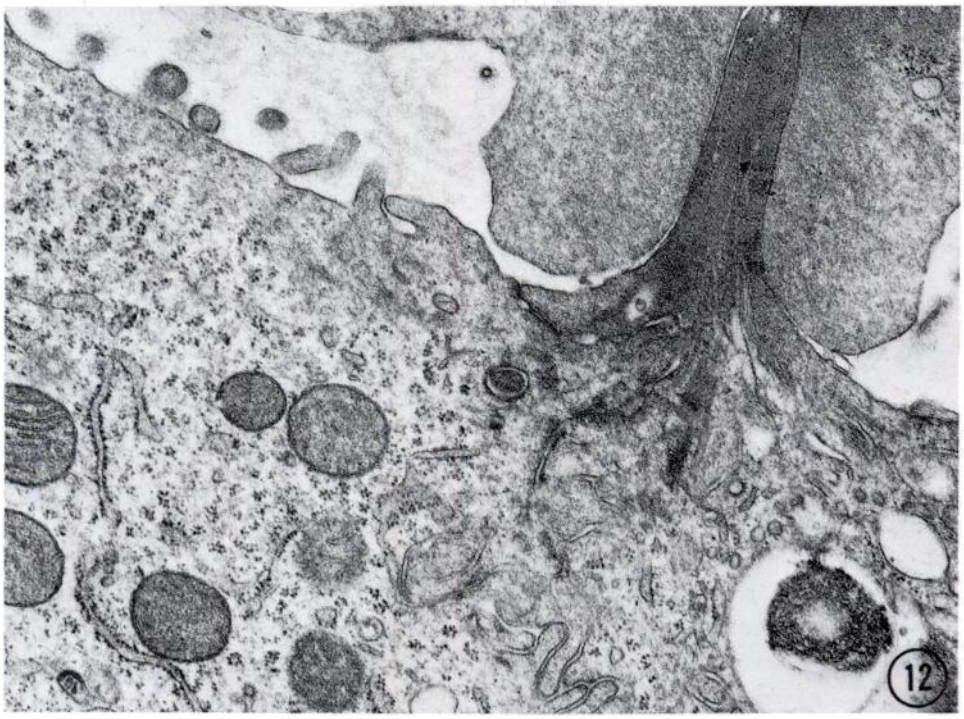


FIG. 11. Ferret implantation site. An ectoplasmic area of syncytial trophoblast contains engulfed fragments of cell (probably uterine), and is closely apposed to uterine surface at the left of the picture. The uterine surface at the right is covered by a thick "glycocalyx" except in that area where it was closely associated with the trophoblast. 12 days p.c. ×14,000.

FIG. 12. The apparently adhesive and phagocytic nature of ferret trophoblast can be seen in this electron micrograph. A portion of uterine epithelial cell cytoplasm has been drawn into the syncytial trophoblast. 13 days p.c. ×28,000.

Reinius. By the usual criteria of light microscopy there appear to be syncytial trophoblast already developed. Moreover, the uterine epithelium is still columnar, and although individual cells show some distortion, there is little evidence of destruction or phagocytosis of uterine epithelial cells. Subjectively, the cellular interactions appear more similar to those of the intrusive implantation mechanism than to fusion of trophoblast with uterine epithelium. Hopefully a closely timed series of implantation stages from the macaque will give us more direct evidence concerning methods of attachment and epithelial penetration, although the epithelial plaque formation somewhat limits its general usefulness as an example of primate implantation.

Displacement Implantation

Some displacement of uterine tissues, including the surface epithelium, by invading trophoblast is a common phenomenon in implantation. However, in a number of species the uterine luminal epithelium appears to be readily dissociated from its underlying basal lamina, and large areas of epithelium are displaced before the trophoblast penetrates beyond the level of the residual basal lamina. In these species (mouse, rat, possibly hamster and vespertilionid bats), the alteration in the integrity of the luminal epithelium allows both individual cells and groups of cells to become free from the underlying basal lamina and from their internal cell associations.

In the mouse and rat, individual sloughed uterine cells are phagocytized by the trophoblast prior to any penetration of the epithelial layer *per se* (Finn and Lawn, 1968). When the epithelium is being displaced, the trophoblast, which remains cellular, not only phagocytizes the sloughed cells but in addition sends projections extending mesometrially between the basal lamina and the overlying uterine cells at the margin of the advancing embryonic pole of the blastocyst. In the abembryonic region where giant cells are forming, the uterine luminal epithelial cells may be displaced from their basal lamina several cells distant from the trophoblast.

Since the loosening of the uterine luminal epithelium occurs after the decidua has begun to form around the epithelium of the implantation chamber, it has been suggested that the isolation of the epithelium from its underlying vascularity contributes to its loss of cohesiveness. However, Finn and Bredl (1973) have recently demonstrated that the breakdown of the epithelium in the rat may be a programmed function of the epithelial cells *per se*, since the uterine epithelium remains intact in the presence of actinomycin D. These authors also comment that the trophoblast cells can penetrate into the epithelium that has been inhibited from its normal sloughing.

In the hamster also it has been suggested that initial penetration of epithelium by processes from trophoblast occurs prior to displacement (Mickelson, 1969). Whether some intrusion of trophoblast precedes the more extensive displacement seen in these myomorph rodents has yet to be determined.

Clearly after the trophoblast has replaced the uterine epithelium on the lateral sides of the implantation chamber, both passive sloughing of uterine cells and intrusion of trophoblast between uterine cells and basal lamina are observed.

It appears unlikely that displacement penetration of the epithelium could occur in situations where the blastocyst is not closely apposed to large portions of the uterine surface. Although the cytology of implantation in bats has not been extensively studied as yet, most of the species show close apposition of blastocyst to the adjacent uterine epithelium in some sort of specialized implantation chamber, either as a portion of the uterus as in the little brown bat, *Myotis* (Wimsatt, 1944, 1975), or a restricted specialized portion of the uterus as in *Glossophaga* (Rasweiler, 1974). In *Myotis* it appears that the uterine epithelium is displaced from its underlying basal lamina over the entire area of the site of penetration before any invasion of the outer trophoblast layer into the underlying stroma occurs (Enders and Wimsatt,

1968). It has been suggested that in the closely related vespertilionid, *Pipistrellus*, implantation is similar to that in the rat and mouse insofar as there is a progressive increase in adhesion of trophoblast to uterine epithelium, followed by dislodgement of uterine epithelium over a large portion of the implantation chamber (Potts and Racey, 1971).

Fusion Implantation

One of the most fascinating and perhaps strangest methods of implantation is that of epithelial penetration by fusion of trophoblast with uterine luminal epithelium. Although it might seem that fusion of two such genetically different tissues is improbable, it has now been found that the rabbit blastocyst uses fusion with individual uterine epithelial cells as a means of first gaining ingress to the underlying endometrial stroma (Enders and Schlafke, 1971).

The process of epithelial penetration starts with the formation of a large number of syncytial trophoblastic knobs in the abembryonic portion of the blastocyst. Projections from these knobs penetrate the extracellular coats and adhere to the apical cell membranes of the underlying uterine luminal epithelial cells. Shortly after this adhesion, it can be seen that pegs of trophoblast of approximately the width of a single uterine cell appear to penetrate to the basal lamina of the epithelium. As development continues, processes from the pegs penetrate through the basal lamina into the stroma and the underlying stromal vessels. During this time there is an increase in size of area of penetration, but the trophoblast retains complete junctional complexes with the adjacent uterine epithelial cells.

The critical stage with regard to epithelial penetration is that of initial formation of the 'peg.' By examining a number of these small pegs at the earliest stage of implantation, we were able to show that the membranes between trophoblast and uterine luminal epithelial apices fuse (Fig. 13), in the process isolating short stretches of membrane including microvilli, and bringing about the confluence of the cytoplasm of the epithelial cell with that of the syncytial knob (Enders and Schlafke, 1971; Enders, 1972). Subsequently the cytoplasm of this 'cell' loses its previous characteristics and is converted into syncytium. Presumably the genetic information transcribed from the entrapped maternal cell is insufficient to maintain the organization of that cell as an epithelial cell in the presence of the more numerous nuclei from the syncytial knob. Curiously, the penetration of the endothelium of the underlying vessels does not appear to involve fusion (Enders and Schlafke, 1971; Steer, 1971).

At the time of formation of the first pegs, the extracellular coats of the blastocyst remain between the rest of the trophoblast and uterine luminal epithelium between pegs. As implantation continues, the material lying between these two epithelia disappears, and eventually there is a more massive fusion between trophoblast and the extensive maternal symplasma formed at this later time (Larsen, 1961).

Why this mechanism of implantation appears in this species is purely speculation. It certainly maintains the integrity of the uterine epithelium during the first stages of implantation. It also tends to aim the trophoblast at the vessels underlying the uterine folds. Böving (1962) showed that the trophoblast relationship is non-random. It rapidly places the trophoblast adjacent to maternal vessels for exchanges in either direction. Although the histocompatibility factors associated with the maternal epithelial cell would be expected to persist in the early stages of peg formation (experimentally fused cells show such factors for a time, Frye and Edidin, 1970), it is difficult to believe that membrane formed subsequently would not display principally the trophoblast characteristics.

Adhesion, Locomotion and Progressive Invasion by Trophoblast

Adhesion of trophoblast to uterine cells is involved in the initial anchoring of blastocyst to the apical surface of uterine epithelium, as

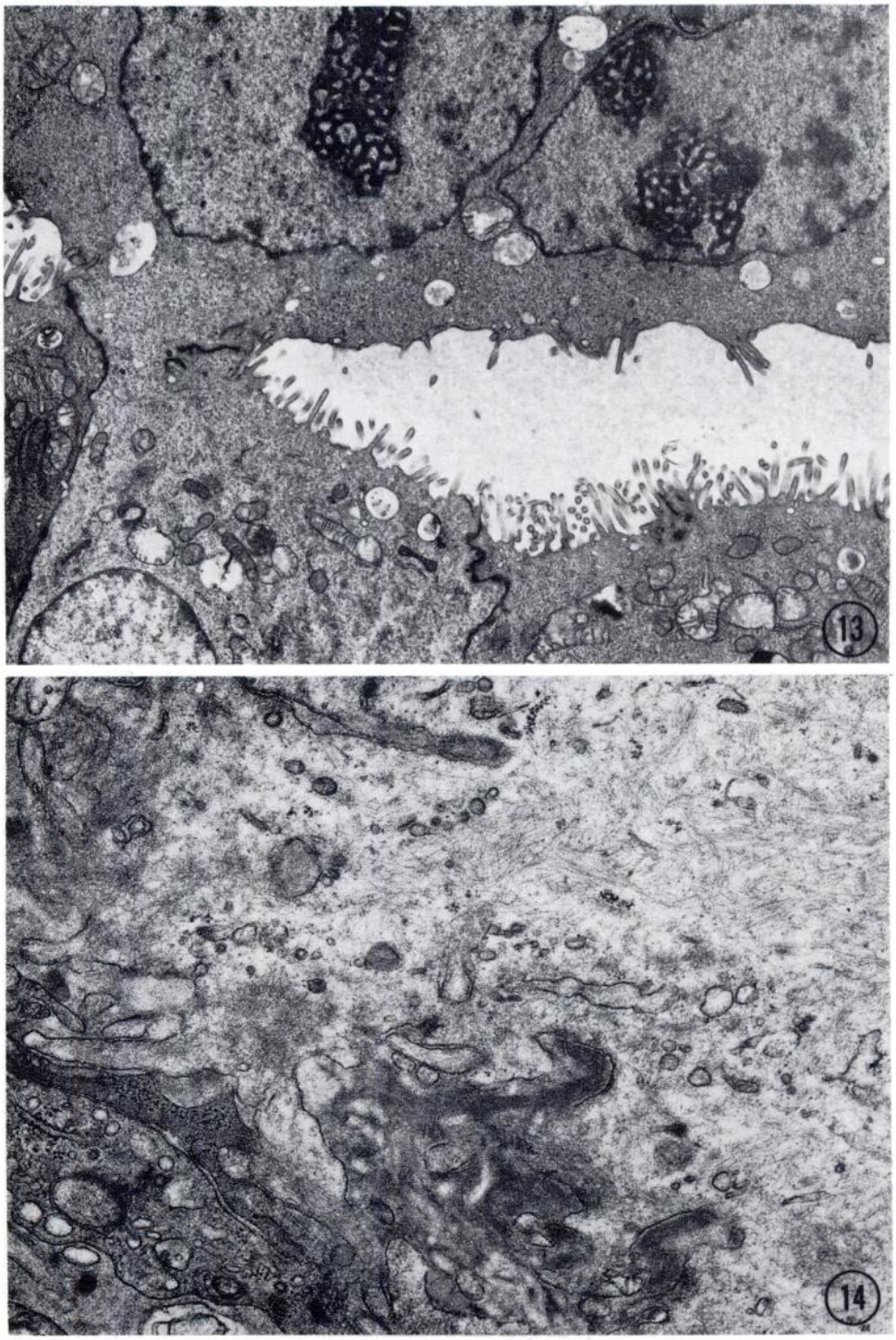


FIG. 13. Rabbit implantation site. The syncytial trophoblast of a trophoblast knob has fused with a single uterine luminal epithelial cell. Note continuity of cytoplasm between the two cells, and the remnants of the cell membranes at the right of the fused area. 7 days 0 hours p.c. ×4,200.

FIG. 14. Syncytial trophoblast of a rabbit at a later stage of implantation. Note the numerous filaments within the trophoblast, and the irregular processes penetrating through the residual basal lamina of the uterine epithelium to a uterine vessel in the lower left. 7 days 18 hours p.c. ×28,000.

was discussed in the previous section. After this epithelium is breached, further penetration involves the flow of trophoblast not only through the epithelium but also through its basal lamina, and into stromal tissues. During the first stages of this process trophoblast appears anchored laterally to intact epithelium. Adhesion in later stages of implantation may be involved in flow of trophoblast cytoplasm into tissues, in surrounding of uterine glands, in penetration into blood vessels, etc.

The specific requirements of adhesion *per se* for cell locomotion, and the role that differential adhesiveness may play, is not always clear (see Wessels, Spooner and Luduena, 1973; Curtis and Büültjens, 1973). Wiseman, Steinberg and Phillips (1972), examining rearrangements of variably adhesive tissues *in vitro*, illustrated that changes in cellular adhesion may initiate morphogenetic movement and influence the pattern of assortment of different cell types. Trinkaus (1973) has suggested that locomotion in *Fundulus* blastula is accompanied by the development of surface projections which subsequently become adhesive, permitting movement of the associated cells which are without contact inhibition.

Invading trophoblast contains variable but often striking ectoplasmic areas. These ectoplasmic regions not only occur where trophoblast is adhering to epithelial cells, but are especially well developed where projections from trophoblast indent the maternal epithelial cells (ferret: Enders and Schlafke, 1972; mare: Allen, Hamilton and Moor, 1973). Both fine filaments and microtubules underlying the cell surface have been implicated in cell motility and contractility in a variety of cell types (Wessels *et al.*, 1971; Luduena and Wessels, 1973). The involvement of microfilaments in the lobulation of salivary gland rudiments is well documented (Spooner, 1973). Cytochalasin is able to block cleft formation in such salivary glands (Wessels, Spooner and Luduena; 1973; Spooner, 1973). In part because of the ability of such filaments to bind myosin in a fashion similar to actin, the filaments are generally considered a major contractile element of the cytoplasm (Adelstein, Pollard and Kuehl, 1971; Bray and Bunge, 1973). Although microtubules (which are abundant in the deeper portions of the ectoplasmic zones of invasive trophoblast) were originally associated with cell movement, more recent work has tended to consider them part of a cytoskeletal framework about which cell movements can proceed (Burnside, 1971, 1973; Porter, 1973).

Nerve growth cones in culture contain peripheral areas of microfilaments and smooth vesicles (Bunge, 1973; Bray and Bunge, 1973). These structures are also characteristic of invasive processes of trophoblast (Figs. 10, 14). Again cytochalasin is an effective agent for arresting the movement of growth cones, and consequently halting the outgrowth of the nerve process. Although it might be difficult to apply such an agent to trophoblast *in situ*, it would be interesting to determine whether trophoblast outgrowth could be temporarily inhibited in rabbit blastocysts removed from the uterus at the time of syncytial knob formation, or whether the outgrowth of trophoblast from rat or mouse blastocysts cultured on collagen could be inhibited.

In all three types of implantation, the trophoblast appears to hesitate in its progress at the level of the residual basal lamina of the uterine luminal epithelium, although this structure does not appear to be an impressive physical barrier insofar as it is thin and does not have well organized collagen fibrils. The pause before the basal lamina is breached might merely represent a time during which the nature of trophoblast growth is undergoing alteration. It could also represent a direct response to the basal lamina, either through a tendency of the trophoblast to adhere to this layer and hence grow along it, to have the motility of its processes inhibited, or other less obvious responses to special properties of this lamina. The role of basal laminas not only in delimiting developing layers in embryonic tissues but as a possible source of direct stimulatory effect is currently the subject of a variety of studies (Hay, 1973). Increased

understanding of the reactions of growing tissues to such laminas may also provide insight into the trophoblast reaction to basal laminas.

CELLULAR ASPECTS OF SECRETION AND ABSORPTION AT IMPLANTATION

Endocytosis and Phagocytosis by Trophoblast

There is appreciable evidence for extensive endocytic activity by the blastocyst prior to and during implantation. Studies using tracer proteins have indicated that trophoblast of both rat and rabbit blastocysts has the capacity for extensive micropinocytosis of exogenous protein introduced into the environment of these blastocysts (Schlafke and Enders, 1973; Hastings and Enders, 1974). Interestingly the rat blastocyst during delayed implantation can ingest large amounts of protein. The mechanism of endocytosis of such materials is by coated micropinocytotic vesicles, with subsequent inclusion into larger vesicles and vacuoles.

As implantation proceeds, there is greater evidence of phagocytic activity. Sloughed individual uterine epithelial cells are phagocytized by trophoblast of the rat and mouse (Finn and Lawn, 1968; Wilson and Smith, 1970). The syncytial knobs of rabbit tro-

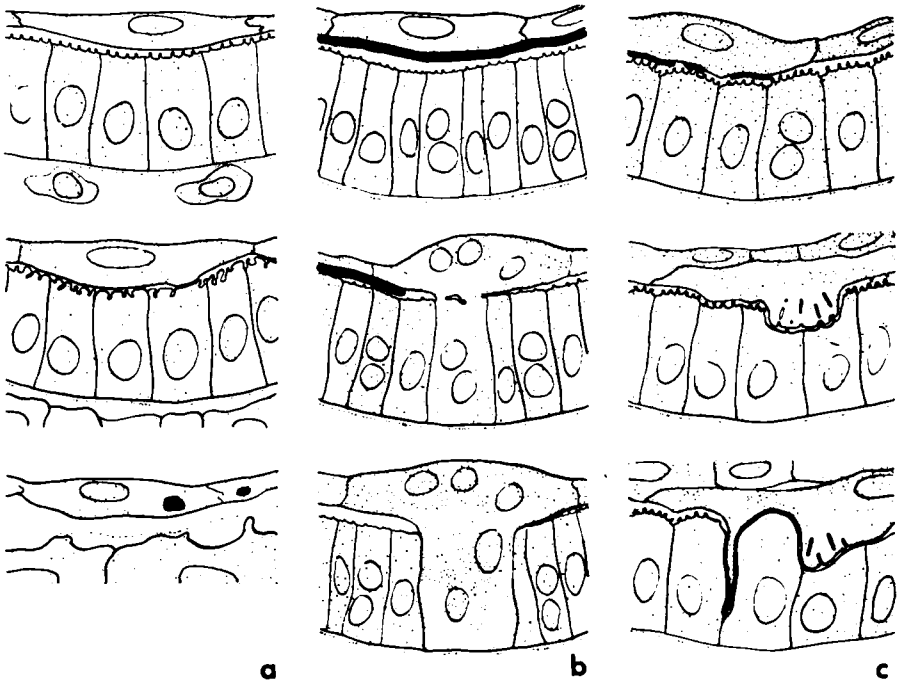


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FIG. 15. Three types of interaction of trophoblast with uterine epithelium during penetration of this epithelium. In all three types, apposition and adhesion precede penetration. However, in *a*, ***displacement penetration*** (rat, mouse), the uterine luminal epithelium is readily dislodged from the basal lamina, and the trophoblast comes to lie along areas previously occupied by the displaced uterine cells. In *b*, ***fusion penetration*** (rabbit), syncytial trophoblast fuses with a uterine luminal epithelial cell. In *c*, ***intrusion penetration*** (ferret, others?), projections of syncytial trophoblast penetrate between uterine epithelial cells.

phoblast and plaques of ferret blastocysts contain numerous vacuoles which rapidly ingest exogenous proteins (Enders, 1971).

It is not surprising that areas of trophoblast which appear adhesive are also capable of phagocytosis and pinocytosis, since adhesion and cell movement are involved in both processes (Figs. 11, 12). Although epithelial cells which become dislodged are phagocytized by adjacent trophoblast, there is little evidence that the position that the cell occupied in the epithelium can be exploited as a point of trophoblast penetration. The sloughed cells are drawn into trophoblast rather than trophoblast progressing into uterine epithelium. Regions of epithelium that become completely surrounded by trophoblast eventually begin to degenerate, suggesting that autolysis plays a major role in their disintegration.

Evidence for Lysosomal Activity

In cytological studies of blastocysts prior to and during implantation, membrane-enclosed material is seen within trophoblast cells. Even in the early blastocyst stage, fragmented material and supernumerary sperm are apparently ingested by trophoblast (Schlafke and Enders, 1967, 1973; Tachi *et al.*, 1970). The localization of acid phosphatase in the Golgi complex and in membrane-enclosed bodies in rat two-cell stages indicates that some of the typical lysosomal enzymes are present early in development (Schlafke and Enders, 1973).

However, with the exception of the work of Abraham, Hendy, Dougherty, Fulfs and Goldberg (1970) on lysosomes in early implantation in the rabbit, most of the studies concerning lytic enzymes have not used electron microscope cytochemical methods. Light microscope histochemical studies of enzymes which are associated with lysosomal activity include those of Christie (1966) and of Smith and Wilson (1971) in the rat and mouse respectively. Acid hydrolases have been demonstrated in the uterine epithelium of the rabbit at the time of implantation (Christie, 1967; Denker, 1972; Kirchner, 1972a). However, their presence (Denker, 1972) or absence (Kirchner, 1972a) in the trophoblast of this species remains somewhat in dispute. In addition, a method using antibodies to endopeptidase has been applied in rabbit blastocysts by Kirchner (1972b). Other methods include that of subjecting synthetic substrates, usually gelatin, to sections of implant sites or explanted blastocysts have been employed by Owers and Blandau (1971) in the guinea pig, Bergstrom (1970) in the mouse, and Denker (1971) in the rabbit.

Since each type of method has its specific advantages and pitfalls, the nature of the procedure tends to influence the interpretation. Light microscope study of frozen sectioned material yields extensive activity, since the freezing process increases the availability of lysosomal enzymes to substrate. Some cellular localization can be achieved, but there is often displacement of relationships during sectioning and it is not possible to resolve most of the objects being lysed. The use of synthetic substrates demonstrates proteolytic activity, but the leaching effect of *in vitro* incubation makes it problematic as to whether the lytic enzymes would be equally available *in vivo*.

In addition to the gelatin substrate method, which can be used to demonstrate both uterine and blastocyst proteases, attempts have been made to demonstrate uterine luminal proteases. Joshi and Murray (1974), using an immunological method, demonstrated formation of a uterine peptidase which they suggested could be involved in sperm capacitation and, because of its appearance on day 5 of pregnancy, in zona lysis. The most imaginatively named material exhibiting proteolytic activity is "implantation initiating factor" (Mintz, 1970). By searching a variety of substrates, Pinsker, Sacco and Mintz (1973) found that this "factor" which can be flushed from the mouse uterus at about the time of zona lysis can digest casein. Further studies will be necessary to determine more specifically the requirement for such a factor in zona lysis.

In summarizing the evidence to date, it is

clear that lysosomal enzymes are present and are involved in both autolysis and phagolysis in trophoblast, and that autolysis is an important feature in the eventual disintegration of uterine cells. It also seems likely that, locally at least, some blastocysts cause a removal of some of the surface materials of uterine epithelium. In addition, proteolytic activity can be demonstrated within the uterine lumen. However, the exact role of blastocyst lytic factors, uterine lytic factors, and local activity has yet to be established either for the process of adhesion or for subsequent epithelial penetration.

Uterine Secretion

Uterine secretory activity has recently received considerable attention. Studies have primarily been focused on whether specific, uterine-created substances are either essential or stimulatory to blastocyst development, and whether there are substances which are inhibitory in nature (Psychoyos, 1973a, b; Weitlauf, 1973). The presence of a specific uterine product in the rabbit has made it possible to study time sequence of formation of this substance (Beier, 1974), and its localization (Daniel, 1972), but has not yet solved the question of whether a specific uterine product is one of the necessary prerequisites to implantation. Secretory activity may be necessary for growth of the blastocyst prior to implantation or for the well-being of the conceptus after implantation, but may not participate in implantation *per se*. Aitken, Burton, Hawkins, Kerr-Wilson, Short and Steven (1973) have pointed out that during the increase in size of the roe deer blastocyst at the end of the period of delayed implantation, there is an increase of secretory material without change in composition of the secretion.

Relatively little attention has been paid to the cellular basis of uterine secretory activity, or to the problem posed by the necessity of obtaining apposition of trophoblast to uterus. Brökelman and Fawcett (1969) described endogenous peroxidase activity within the endoplasmic reticulum of estrogen-treated rat uterus. Enders and Nelson (1973) reported preliminary observations on the occurrence of endogenous peroxidase in apical vesicles of rat luminal epithelial cells at the time of implantation. However, thus far the classical use of labeled precursors in a timed series of autoradiographic materials has not been used to study uterine secretion.

During the apposition stage in all species studied so far, there is some extracellular material interposed between microvilli of the uterus and the overlying trophoblast. However, as adhesion progresses, not only is the space between the adhering membranes of trophoblast and uterus devoid of apparent extracellular material, but in at least three species (rat, ferret, rabbit), the larger spaces lying between trophoblast and uterus away from the regions of adhesion are also cleared of extracellular materials. During delayed implantation in the rat and mouse, blastocysts are tightly clasped by the uterus; consequently neither secretion nor transudation can be occurring in amounts producing accumulation in the uterine lumen at this time.

Evidence for Absorption by Uterus

Vokaer and Leroy (1962) first drew attention to the evidence that rat uterine epithelium could absorb materials, in this case Trypan blue, from the lumen. Psychoyos and Mandon (1971a, b) renewed interest in this aspect of uterine cell biology by their description of "sea anemonae" shaped structures on individual uterine luminal epithelial cells, and suggested that these structures were involved in the removal of uterine fluid. Nilsson (1966, 1972) examined similar structures in the mouse uterus using transmission and scanning electron microscopy, but suggested that they were involved in secretion of materials into the uterine luminal fluid.

Correlation between the structures seen in scanning electron microscopy with pinocytotic activity has recently been established by use of tracer techniques (Enders and Nelson, 1973; Parr and Parr, 1974). Exogenous proteins as well as other markers are engulfed

from the uterine lumen into large ectoplasmic flanges, termed pinopods (Enders and Nelson, 1973). The ingested vacuoles are drawn into the apex of the uterine cell. Subsequently, the material is seen in multivesicular bodies, and is apparently degraded by the lysosomal system of the cell, although some is moved laterally into the intercellular space.

The time of appearance of the pinopods suggests that they function in removal of uterine luminal fluid during apposition, but what other aspects of the blastocyst-uterine relationship they may facilitate are not yet known.

SYNCYTIUM FORMATION

Evidence for Syncytium Formation

Fusion of individual trophoblast cells to form a syncytium is a prominent feature of implantation and placentation, especially in those species that have a hemochorial or endotheliochorial chorioallantoic placenta. However, the time of syncytium formation, the extent and position of syncytium, and how much of the trophoblast it involves are variable. The situation is further complicated by the problem that a number of features associated with syncytium can be discerned by light microscopy, but the presence or absence of cell membranes *per se* can be definitively determined only by electron microscopy.

There is a general pattern of relatively early formation of syncytium in those species in which there is a rapid progression of trophoblast into the endometrium. In the guinea pig, the zona pellucida still surrounds the blastocyst when syncytium formation begins in the thickened abembryonic trophoblast that forms the implantation cone (Blandau, 1949, 1961). Electron microscopic examination of this implantation cone reveals not only the confluence of cytoplasm between several nuclei but in addition evidence that it is produced by cell fusion (Enders, 1971). The most obvious evidence of cell fusion is the presence of segments of various lengths of cell membrane which maintain the standard intercellular distance but are fused at their margins, thus isolating within the cytoplasm a small bit of former intercellular 'space'. Typical desmosomes are commonly included within these remnants (Enders and Schlafke, 1965, 1969). An additional feature that appears to be less obviously related to the fusion is a series of patches of infoldings of the cell membrane, generally situated at the blastocyst margin of former cell junctions. The precociously formed syncytium produces ectoplasmic projections that both penetrate the zona pellucida and adhere to uterine luminal epithelium (Enders and Schlafke, 1969; Parr, 1973).

It is generally assumed that human and macaque blastocysts form syncytium at approximately the time that they are penetrating the uterine epithelium (Heuser and Streeter, 1941; Blandau, 1972). However, the only early implantation stage of the macaque blastocyst which has been examined with electron microscopy failed to demonstrate syncytium formation (Reinius, Fritz and Knobil, 1973). The species we have studied show syncytium formation at progressively later stages in implantation. An adhering armadillo blastocyst showed no evidence of syncytium formation, but two blastocysts that dislodged uterine epithelial cells when they came free of the uterus and all subsequent implantation stages show syncytium formation in trophoblast adjacent to the embryonic cell mass (Enders, 1962, 1964).

The rabbit blastocyst forms a number of syncytial trophoblastic knobs on its abembryonic surface prior to implantation (7 days post coitus) (Böving, 1962). In addition to occasional fused remnants of the cell membranes in rabbit trophoblast knobs, peculiar infoldings of the cell membrane similar to those in the guinea pig are seen on the side of the knobs toward the blastocyst cavity (Enders and Schlafke, 1971; Steer, 1971). The ferret also forms syncytium at the time of implantation, which is 12 days post coitus. However the syncytial plaques of the ferret blastocyst are relatively thin and are not segregated into knobs as they are in the rabbit (Enders and Schlafke, 1972).

In the little brown bat, the trophoblast which will first form syncytium can be seen as a separate layer by the end of uterine epithelial penetration, but it remains cellular until approximately the stage of surrounding the maternal vessels (Enders and Wimsatt, 1968; Enders and Schlafke, 1969). At this time there is extensive conversion of the outer layer of trophoblast to syncytium over the entire area of the chorioallantoic placenta. In the rat and mouse, syncytium formation in relationship to implantation process is even later, and the layer of trophoblast immediately adjacent to maternal blood never becomes syncytial. The other two layers of trophoblast associated with the chorioallantoic placenta eventually become syncytial but apparently relatively late in placenta formation (Jollie, 1964; Enders, 1965).

As has already been suggested, syncytium formation usually occurs in relation to that part of the blastocyst which is invading the endometrium early or, if the syncytium formation is late, it occurs in the chorioallantoic placenta, in that region which forms the closest association with the maternal blood vascular system. Although the implantation cone involves a complete segment of the antimesometrial end of the guinea pig blastocyst, most of the other species having syncytium formation have more patchy distribution. At the present time there is little evidence that any localized aspect of the uterus affects syncytium formation by the blastocyst, at least in the early stages of implantation. It remains possible, however, that the proximity of maternal blood promotes syncytium formation in the chorioallantoic placenta at later stages.

Syncytium is generally a 'terminal tissue', lacking nuclear division and incapable of reverting to cytotrophoblast (Wimsatt, 1945; Galton, 1962). It is probable that, like myotube formation, syncytial trophoblast forms from the fusion of cells in G_1 that are subsequently inhibited from entering the S stage of the cell cycle (Bischoff and Holtzer, 1969). Trophoblastic giant cells of the rat and mouse, on the other hand, are not formed by cell fusion but rather by internal replication of DNA, and should not be considered syncytial in nature (Sherman, McLaren, and Walker, 1972).

Advantages of Syncytium Formation

Syncytium appears to have characteristics which might be advantageous in the implantation process. It apparently has good surface flow characteristics in that not only is it capable of forming ectoplasmic projections but in addition generally shows a great deal of evidence of endocytic activity. The involvement of a large mass of cytoplasm may facilitate the flow of such syncytium into irregular spaces, as well as allowing it to completely surround individual cells, glands, etc.

Syncytium offers a broad face resistant to fragmentation by proteolytic enzymes, including those that it may itself synthesize. It may be a means of isolating cell surface characteristics to a specific part of the trophoblast, for example, the unusual ability of trophoblast to adhere to the apical ends of epithelial cells. Finally, as a terminal tissue, the syncytium exposed directly to the maternal system is less likely to colonize inappropriate areas when unaccompanied by cytotrophoblast than would be a metaplastic layer.

TROPHOBLAST-UTERINE INTERACTION AT A DISTANCE

The preceding consideration of implantation has been confined principally to the early stages of contact of trophoblast with uterine epithelium. There are many other interactions initiated in implantation that are worthy of consideration, including decidua formation, epithelial plaque formation in primates, and the endothelial cell hypertrophy and differentiation in carnivores. In addition, morphological studies of implantation tend to emphasize the effects of trophoblast on uterus, since the uterus can be studied with and without blastocysts. On the other hand, it is more difficult to analyze the effects of uterus on the blastocyst. Some progress has been made in use of *in vitro* models to pro-

duce various types of simulation of implantation (for example trophoblast outgrowth in the mouse and rat). If the ways in which such *in vitro* models resemble and differ from the normal condition are kept in mind, some aspects of trophoblast function and differentiation may be clarified.

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