# Embryo Implantation and Trophoblast Invasion

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ABSTRACT

The trophoblast of the implanting mammalian embryo receives interest for being a highly invasive non-tumor tissue. This becomes particularly obvious when trophoblast is transplanted to ectopic sites.

The physiological sequence of events during implantation of the embryo in the uterus involves apposition, dissolution of extracellular blastocyst coverings, physicochemical changes in the cell surface coats of trophoblast and uterine epithelium, adhesion, and, in most species, penetration of the trophoblast through the uterine epithelium towards subepithelial blood vessels. Various modes of penetration (displacement, cell fusion, intrusion) are described. Many observations suggest that the relation between invasiveness of the trophoblast and readiness of the uterine epithelium to degenerate varies from one species to the other.

The mechanism of the interaction between trophoblast and uterine epithelium which leads to implantation initiation is still largely unknown in spite of intensive research efforts. Recently evidence has been found for an important role played by certain proteinases of these tissues. In the rabbit, a peculiar proteinase of the implanting trophoblast (called "blastolemmase") seems to be essential for implantation initiation. Preliminary biochemical characterization has been achieved. Specific proteinase inhibitors interfere with implantation when administered in vivo. The possible role of this proteinase and of related enzymes in attachment and/or invasion is discussed.

KEYWORDS

Embryo implantation; trophoblast; adhesion; epithelial penetration; invasion; glycoproteins; blastolemmase; control by inhibitors.

## INTRODUCTION

Implantation is the process by which an intimate cellular contact is formed between embryonic and maternal tissues, as typically found in eutherian mammals. In histological terms, the involved partners are: 1. the trophoblast, i.e. a specific

extraembryonic population of cells of the conceptus which appears to be specialized for the formation of this contact (and, besides this, for nutrition and hormone production), and 2. the endometrium of the uterus. In most species including the human, implantation involves true invasion of the trophoblast into the endometrium. The cytological details of this process are of interest because they resemble, in various respects, tumor invasion. The destructive potential of the trophoblast becomes particularly obvious when these cells are transplanted to ectopic sites, as described below. However, in the hormonally conditioned uterus is trophoblastic invasion limited in time and space and is halted before causing total destruction of the host organ. Instead, a complex morphogenetic process which involves both partner tissues is initiated at the border between them, leading to the formation of a highly specialized exchange organ, the placenta.

We will consider here only those species in which a truly invasive placenta is formed, omitting the epitheliochorial placentation found e.g. in ungulates where trophoblast and uterine epithelium are only tightly apposed against (and adhere to) each other. Interestingly, it has been reported that even in these latter species shows the trophoblast invasive properties if transplanted to ectopic sites (Samuel, 1971).

## CYTOLOGICAL DETAILS OF THE INTERACTION BETWEEN TROPHOBLAST AND ENDOMETRIUM

Recently attention of investigators is focused on the initiation phase of implantation. One of the most astonishing phenomena in this process is its initiation by formation of a cellular contact between the apical parts of cytoplasm of the two epithelia , the trophoblast and the uterine epithelium. Implantation then continues by penetration of the trophoblast through the uterine epithelium followed by invasion into the deeper parts of the endometrium. The cytological details to be described below are found quite consistently and seem to be largely independent of the type of topographical relationship between the blastocyst and the uterus present in the particular species at the time of implantation, i.e. the centric (large blastocyst which fills the uterine lumen), the eccentric (small blastocyst which implants in an endometrial crypt which it fills first) or the interstitial type (small blastocyst which penetrates, as a whole, through a minor defect in the uterine epithelium). In particular, there is no consistent correlation between these three types of topographical relationship and the three modes of epithelial penetration to be described below.

Electron microscopical studies performed in a number of species during the last years have shown that there is a certain rule in the sequence of cytological details seen during the initial phase of implantation (for review, see Schlafke and Enders, 1975). In the first phase, a stable cellular contact is formed between the blastocyst, which was before freely movable in the uterine lumen, and a portion of the uterine epithelium. This phase consists of two stages, apposition and adhesion.

During apposition, the blastocyst becomes immobilized and those parts of the endometrium which will enter into interaction with the trophoblast are determined. The mechanism of this process and the morphological details vary considerably from one species to the other. In species with small blastocysts (rodents), a local oedema induced in the endometrium in the vicinity of the blastocyst is involved in the immobilization of the embryo, whereas myometrial contractions play a significant role in species with large blastocysts (rabbit, see Böving, 1963).

At this stage, the blastocysts are still surrounded in a number of

species by thick coats of extracellular material, the so-called blastocyst coverings, i.e. the zona pellucida or more complex, multilayered structures (as seen in the rabbit and the fur seal) (Böving, 1963; Denker, 1970, 1977; Denker and Gerdes, 1979). There is evidence that physicochemical changes which these coverings undergo in this phase play a role in the process of apposition: the adhesiveness of the coverings increases considerably (especially at the abembryonic pole, i.e. where implantation begins, as shown in the rabbit). It has been postulated that this local change in adhesiveness is involved in establishing the correct orientation of the blastocyst in the uterus, with its abembryonic (trophoblastic) pole facing the antimesometrial endometrium (Böving, 1963; Denker, 1978). Enzyme activities probably involved in this process will be discussed below.

The increase in adhesiveness is directly followed by dissolution of the blastocyst coverings, and the trophoblast establishes a cellular contact with the uterine epithelium immediately thereafter, in the rabbit. This close sequence of events has found interest because there might be a connection between the causal mechanisms. The same is possibly valid also for other species (e.g. the ferret) including also the murine rodents (mouse, rat etc.) although there has been much confusion in the literature, because in the latter group the blastocysts are able to hatch mechanically from their zona pellucida and to remain unimplanted in the uterine lumen, as seen during lactation-induced delay of implantation. However in regular pregnancy, the described sequence of events is found quite typically (for references, see Denker, 1977). In several other species (cat, ungulates) the dissolution of blastocyst coverings and formation of a cellular contact between trophoblast and endometrium are always clearly separated in time. The situation in the human remains uncertain because specimens from the apposition and attachment phases are lacking, and the investigated unattached human blastocysts have been fixed with solutions which are unsuitable for preservation of the coverings. In the guinea pig which shows an interstitial implantation like human embryos, ectoplasmic processes of trophoblast cells penetrate through the zona which is apparently dissolved by proteolytic enzymes; immediately afterwards they establish contact with the uterine epithelium (Spee, 1901; Blandau, 1949; Parr, 1973).

After the blastocyst coverings have been disposed of, the trophoblast approaches the surface of the uterine epithelium, and the microvilli of both epithelia interdigitate showing a more or less regular pattern, depending on the species. Even at this stage of apposition, the blastocysts can still be flushed out of the uterus using slight pressure, although a certain degree of adhesion has doubtless been reached.

The morphologically observable intimacy of the contact between both epithelia is thereafter gradually increased. In terms of electron microscopical classification the adhesion stage is reached when the apical cell membranes of trophoblast and uterine epithelium not only approach each other focally but run parallel to one another over longer distances, and there are regions of very close (less than 200 Å) membrane association. The microvilli of both epithelia which had before shown regular interdigitation as described above flatten, and the parallel running cell membranes form an irregular, waved contour (Enders and Schlafke, 1967; Reinius, 1967; Bergström, 1971; Parkening, 1976). This sequence of events is found more or less typically in all species investigated so far. In some species, however, only certain parts of the trophoblast establish the contact mentioned. In the rabbit, for example, the initial contact is formed only by specialized elements of the trophoblast, the syncytial "trophoblastic knobs" (Böving, 1963; Denker, 1970, 1977). In the murine rodents, the adhesion takes place fairly uniformly over the entire trophoblast surface. The two sets of epithelial cells which have established contact in the described way show little cytological specialization except for prominent ectoplasmic regions which are often rich in microfilaments.

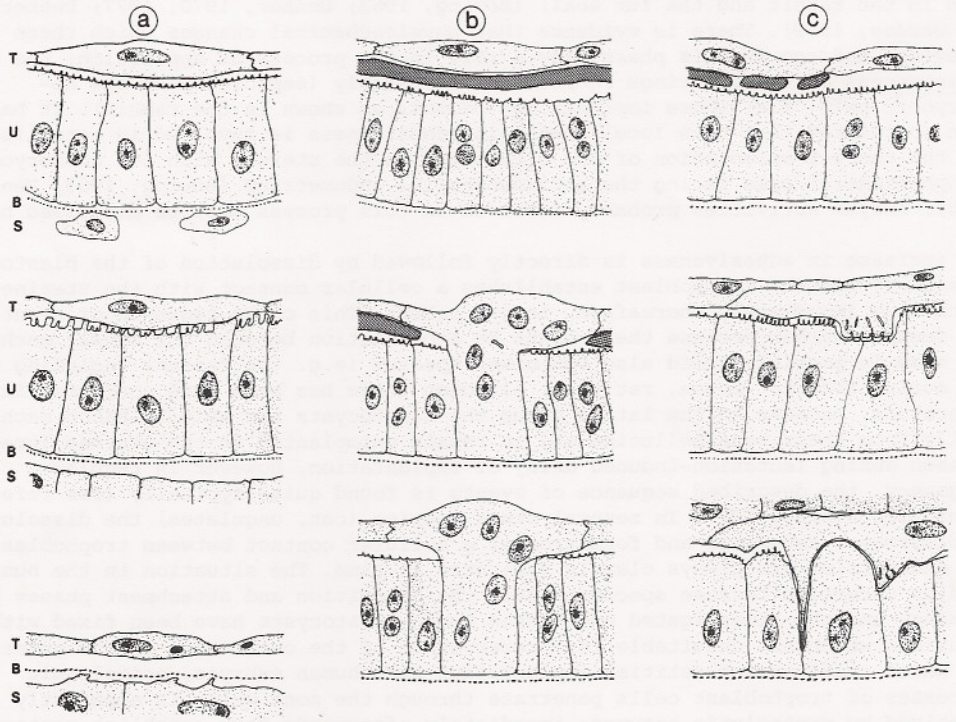



Fig. 1. The three types of interaction of trophoblast with uterine epithelium during the apposition, adhesion and penetration phases of implantation. (a) Penetration by displacement (rat, mouse); (b) penetration by fusion (rabbit); (c) penetration by intrusion (ferret, other species?). (Adopted, in modified form, from Schlafke and Enders, 1975, by permission of the authors and the editor, Biology of Reproduction)
T: trophoblast; U: uterine epithelium; B: basal lamina; S: stroma cells being transformed into decidual cells in (a).

The cellular contact is reinforced in the beginning of the next phase, epithelial penetration. Typical junctional complexes including even desmosomes are formed between the invading trophoblast and the uterine epithelium. This phenomenon has received interest since it is known that, apart from formation of mechanical contact, certain junctions can mediate ionic coupling and information transfer which seems remarkable when it occurs between two epithelia of different organisms, the embryo and the mother.

A comparative analysis of the cytological details observed in various species has shown that three different types of epithelial penetration can be distinguished as illustrated diagrammatically in Fig. 1 (Schlafke and Enders, 1975):

1. Penetration by displacement: In a number of species (like murine rodents), the uterine epithelium is sloughed off the basal lamina in relatively large areas adjacent to the implanting blastocyst. By this way the trophoblast can come into contact with the denuded basal lamina, and it then grows between the latter and neighboring epithelial cells thus apparently contributing to the process of displacement. Sloughed epithelial cells are found both in groups and as individual cells, and are phagocytized by the trophoblast. Numerous investigations have well documented that the uterine epithelium has, in these species, a pronounced tendency to undergo the described sloughing, even after nonspecific mechanical stimuli. There is some evidence that this tendency may be a peculiar property of the uterine epithelial cells themselves, preprogrammed under proper hormonal stimulation. It may require specific genetic activity because the epithelium remains intact after actinomycin D treatment (Finn and Bredl, 1973). There is also evidence that the loss of the epithelium partly depends in addition on isolation from the blood supply by differentiation of the decidual cells which form a "barrier" under the basal lamina (Fig. 1, a). Formation of decidual cells is in fact induced by the same kind of stimuli (blastocyst, mechanical alteration) mentioned above. Interestingly, the actinomycin experiments have shown that the trophoblast does have the ability to penetrate into epithelium which appears to be intact, even in these species. Displacement penetration, therefore, may be a very special situation, and regular implantation in the uterus as seen in these species may not be a very suitable model for studies of trophoblast invasiveness.

2. Penetration by fusion: Initiation of implantation by fusion of trophoblast with uterine luminal epithelium has been well documented electron microscopically in the rabbit (Enders and Schlafke, 1971). Material from primates and the human as investigated so far leaves some doubt whether fusion might also take place in these species (Böving and Larsen, 1973; Schlafke and Enders, 1975). In the rabbit, syncytial elements of the trophoblast, the so-called trophoblastic knobs, penetrate through the extracellular blastocyst coverings, adhere (as described above) to the surface of uterine epithelial cells which are overlying a subepithelial capillary (Böving, 1963), and, immediately thereafter, fuse with them so that a compound symplasm is formed. The fate of the maternal nuclei in this symplasm is not known exactly, although there is no indication of degeneration. In any case, the fact that the number of trophoblast nuclei in this early fusion stage is larger than that of uterine nuclei appears to be sufficient to induce a change in cellular behavior so that the basal lamina will be penetrated and the subepithelial capillary be arroded until a hemochorial contact is formed (see Denker, 1977: Fig. 4). The trophoblast is particularly rich in microfilaments, and it should be interesting to know more about the role of these structures and of microtubules in the process of invasion. The description just given is based on the details seen during formation of the yolk sac placenta, but basically the same phenomena are observed at the formation of the chorioallantoic placenta which follows, with the only difference that both the uterine cavum epithelium and the trophoblast are first transformed into broad symplasms, which then show extensive fusion (Larsen, 1961).

3. Penetration by intrusion: This type of epithelial penetration has been documented best in the ferret (Enders and Schlafke, 1972). Histological evidence from other species which have been studied only or predominantly with the light microscope suggests that intrusion penetration may be found in many species (including the guinea pig and possibly primates ). In the ferret, the trophoblast of the implanting blastocyst first forms syncytial elements which are comparable to the trophoblastic knobs seen in the rabbit. Ectoplasmic pads of syncytial trophoblast attach to the surface of the uterine epithelium and often indent it. This is followed by penetration of thin processes of the trophoblast between the apical ends of uterine peithelial cells. Interestingly, these parts of the trophoblast then share the apical junctional complexes of the uterine epithelium, and a number

of desmosomes are formed between both partners. Subsequently, the trophoblast processes traverse the basal lamina and rapidly invade the stroma where they surround the blood vessels forming the endotheliochorial contact typical for carnivores. The described trophoblast processes are rich in microfilaments and also possess microtubules.

Trophoblast invasion seems to be halted for a while before penetrating through the basal lamina, in all three types of implantation. The mechanism of overcoming this barrier is unknown.

The following phase of deeper penetration into the endometrial stroma is quite impressive from the histological point of view because the invasive properties of the trophoblast become perhaps even more obvious. The intimacy of contact finally reached is traditionally described using Grosser's classification (endotheliochorial and hemochorial contact being the principal types seen in invasive placentae) although this contributes little to understanding the cell physiological properties of the trophoblast. Purely desciptive morphological studies of implantation as seen in regular pregnancy have generally given only very limited information to those interested in the mechanisms of invasiveness.

## ASPECTS OF ECTOPIC TROPHOBLAST GROWTH AND THE ROLE OF HOST TISSUE IN REGULATION OF INVASION

When transplanted to extrauterine (ectopic) sites, the trophoblast shows its invasive potential most impressively. Ectopic growth of the trophoblast has been studied extensively in the mouse where it can easily be obtained after transplanting preimplantation stage embryos to such sites as the kidney or the testis (Kirby, 1970; for review see Billington, 1971). In this situation it becomes particularly obvious that trophoblast invasion is accompanied by destruction and phagocytosis of host tissues, although these phenomena form also part of the regular implantation process in the endometrium. It was shown that transplanted trophoblast can cause destruction even of mouse mammary carcinoma (Kirby, 1962). The type of host tissue seems to determinate, at least to a certain extent, whether cytolysis at distance dominates (as seen in the testis) or whether vital appearing host cells are engulfed and phagocytosed. In the latter case, cell junctions including desmosomes can be formed between trophoblast and host cells (in the same way as in regular uterine implantation, see above) as described in case of human choriocarcinoma growing in hamster liver.

A widely favored hypothesis postulates that trophoblast invasion in the uterus is regulated by the decidua which may function as a barrier, possessing inhibitory properties of unknown nature. In fact, trophoblast transplanted into a non-decidualized uterus (lacking proper hormonal conditioning) in the mouse causes marked destruction of the host organ up to the level of the myometrium. This may be comparable to the situation of placenta accreta in man. It has, therefore, been suggested that, in regular pregnancy, the extent of trophoblast invasion is determined by the limits of an area in which the decidual cells have a tendency to degenerate even spontaneously (for review, see Billington, 1971; Finn, 1971). On the other hand, sarcoma cells transplanted to the hormonally conditioned uterus were found to invade right through the decidua to the myometrium (Smith and Hartman, 1974). Furthermore, the example of ectopic implantation shows that the trophoblast seems to have an inherently limited life-span: the invasive growth of extrauterine trophoblast lasts only a few days longer than the time at which it normally ceases in the uterus, and it finally degenerates at approximately the same time at which pregnancy would be terminated. It may be significant that invasion stops before degeneration of trophoblast cells becomes apparent (Sherman and Wudl, 1976), and this may indicate that proliferation and invasion are not

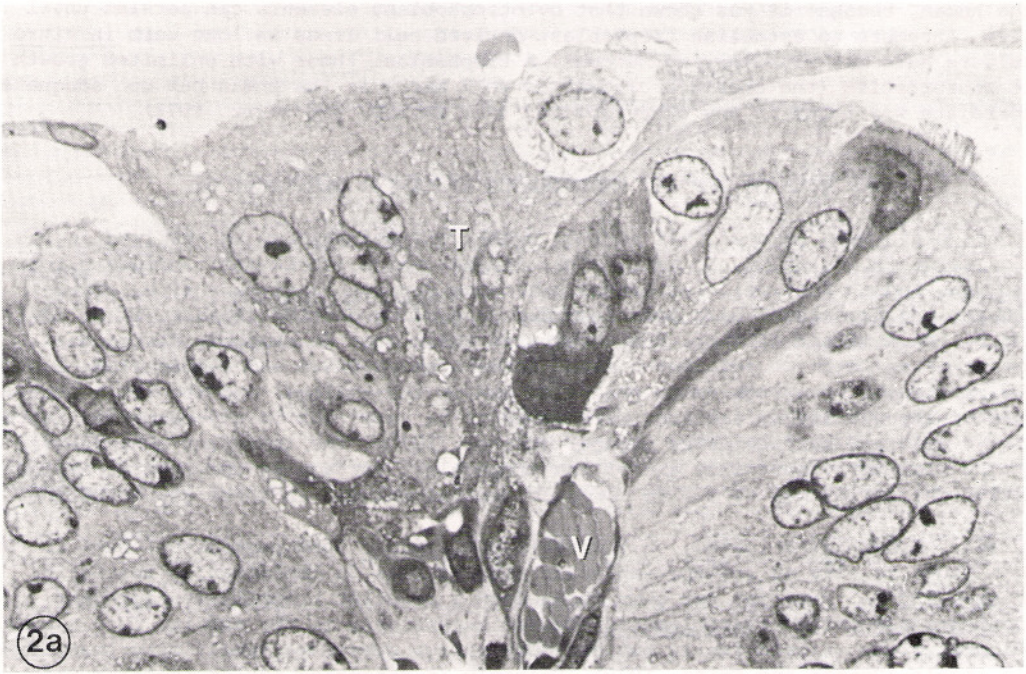
necessarily connected. Anyway the stop of invasion cannot simply be explained by disappearance of cytotrophoblastic "stem" cells as suggested for the situation in the human, because it was shown that cytotrophoblast elements can persist until term. Attempts to establish trophoblast-derived cell lines in long term in vitro culture have met with varying success. A trophoblast tumor with unlimited growth characteristics (the choriocarcinoma) is well known in the human but no adequate correlate was found in any other species (for review, see Hertz, 1978).

## THE ROLE OF CERTAIN PROTEINASES IN EMBRYO IMPLANTATION

It has often been assumed that various hydrolytic enzymes may be involved in the process of implantation. In particular, enzymes which attack cell surface glycoproteins and/or intercellular ground substance glycosaminoglycans (i.e. glycosidases and proteases) have been regarded as good candidates (Denker, 1970, 1971 a and b). As a matter of fact, the structures which must be overcome during formation of an intimate cellular contact between trophoblast and endometrium, i.e. the blastocyst coverings (zona pellucida and/or its analogs) and the thick surface coat of the uterine epithelium, were found to be very rich in glycoproteins (with sialic acid and sulfate ester groups) (Denker, 1970). The increased adhesiveness of the blastocyst coverings and of the trophoblast surface as observed at implantation initiation may be due to physicochemical changes in these glycoproteins.

Of all enzymes investigated, only certain proteinases (endopeptidases) have so far been shown to play an indispensable and crucial role in implantation initiation (Denker, 1977). Interest has been focused on a peculiar trophoblast-dependent endopeptidase called blastolemmase studied extensively in the rabbit. This enzyme belongs to the trypsin family but has more narrow substrate specificity than trypsin itself (Denker and Fritz, 1979). Probably in concerted action together with uterine secretion proteinase(s) (and perhaps with glycosidases also shown to be present), blastolemmase appears to play a central role in initiation of implantation. When rabbits are treated intrauterally with specific proteinase inhibitors (which were selected on the basis of blastolemmase inhibition in vitro), the dissolution of the blastocyst coverings is blocked and the trophoblast cannot attach to the uterine epithelium (Fig. 2 and 3) (Denker, 1977). Inhibitors studied were: aprotinin (Trasylol), antipain, boar seminal plasma trypsin-acrosin inhibitor, and p-nitrophenyl-p'-guanidinobenzoate (NPGB). On contrast, epsilon-aminocaproic acid, an inhibitor of plasminogen activation which does not inhibit blastolemmase, is without any effect on this process. While it became clear from the described experiments that blastolemmase plays an indispensable role in the dissolution of blastocyst coverings as part of the processes leading to implantation, it is not certain at the present time whether this enzyme is directly involved in initiation of attachment of the trophoblast to the uterine epithelium and its subsequent fusion and invasion: In inhibitor-treated uteri, occasional attachment and fusion, although restricted to few small areas, was observed in regions where interposed blastocyst coverings were shed due to mechanical rupturing as a result of the continuing expansion of the blastocyst. Experiments presently being performed in this laboratory will hopefully answer this question.

It should be interesting to have comparative data from other species in which far less is known about the possible role of proteinases in implantation. Trypsin-like enzyme(s) seem(s) to be essential for implantation in the mouse (for review see Denker, 1977). In the cat, a cathepsin B-type enzyme predominates, the physiological function of which is unknown (Denker and coworkers, 1978). An enzyme with trypsin-like activity was also found in implanting blastocysts in the guinea pig (Denker, unpublished). The question whether this is related to the gelatinolytic activity found at the same stage in this species (Blandau, 1949) will have to be answered by further experiments.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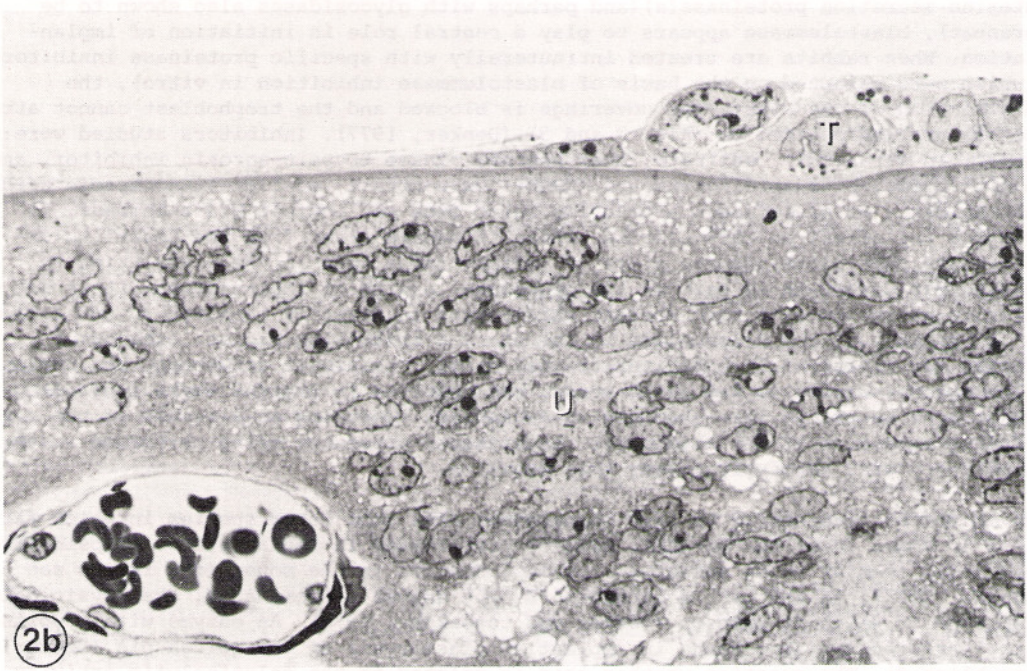
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In addition to blastolemmase, trypsin family enzymes which have even narrower substrate specificity are present in implanting rabbit blastocysts (Denker and Petzoldt, in preparation). Such enzymes might be involved in changing, by limited proteolysis, cell surface properties (including adhesiveness and receptor functions) relevant to attachment and invasion. This aspect is being studied in recent experiments.

ACKNOWLEDGEMENT

The author wishes to thank cordially Mrs. Gerda Bohr and Edith Höricht for excellent technical assistance, Mrs. Ria Becht for typing the manuscript, Mr. W. Graulich for drawing the diagram,and to Drs. Sandra Schlafke and A.C. Enders for the permission to use their illustration (Fig. 1). Author's own investigations were supported by Deutsche Forschungsgemeinschaft grants No. De 181/3-8.

Fig. 2 (Opposite page) Light micrographs of semithin sections illustrating inhibition of embryo implantation by administration of proteinase inhibitors in the rabbit. x 1000.

a) Control, 7 1/2 d p.c. The syncytial trophoblastic knob (T) has penetrated the uterine epithelium and has already nearly reached the subepithelial blood vessel (V).

b) Proteinase activity was inhibited by administration of 6 mg of aprotinin (Trasylol) into the uterine lumen at 6 1/2 d p.c. The stage shown is 8 1/2 d p.c., i.e. one day later than the control (a). Even at this time, the blastocyst coverings (dark line) are not dissolved yet and are interposed between trophoblast and uterine epithelium (U) (already transformed into a broad symplasm). The trophoblastic knob (T) has not been able to attach, to invade and to reach the subepithelial blood vessel (lower left corner).


Fig.3. Electron micrograph showing the extracellular glycoprotein material of the blastocyst coverings (B) still interposed between rabbit trophoblast (T) and uterine epithelium (U) at 8 1/2 d p.c. Proteinase activity was inhibited by intrauterine administration of 6 mg of aprotinin (Trasylol) at 6 1/2 d p.c. Even 1 1/2 days after the regular time of implantation, no cellular contact has been established between trophoblast and uterine epithelium. The apical part of the latter has formed hemidesmosome-like structures where it touches the blastocyst coverings. Bar = 1 µm.

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# CELL MOVEMENT AND NEOPLASIA

Proceedings of the annual meeting of the Cell Tissue and Organ Culture Study Group, held at the Janssen Research Foundation, Beerse, Belgium, May 1979

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First edition 1980

**British Library Cataloguing in Publication Data**

Cell Tissue and Organ Culture Study Group. *Annual Meeting, Beerse, 1979*
Cell movement and neoplasia.
1. Cancer cells - Congresses
2. Cells - Motility - Congresses
I. Title II. Brabander, Marc de
III. Mareel, M IV. Ridder, L de
616.9'94 RC269 79-42740

ISBN 0-08-025534-5

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