BASIC ASPECTS OF OVOIMPLANTATION *

H.-W. DENKER

Implantation of the blastocyst in the uterus is not only of fundamental importance for the estabishment of pregnancy, but it is also a biologic phenomenon of tremendous fascination. Recent progress in implantation research appears to open some ways on how to manipulate this process, particularly during its initial phase.

For heuristic reasons, it seems appropriate to use a narrow *definition of implantation,* i.e., the specific process that leads to the formation of a specialized, intimate cellular contact between the trophoblast of the embryo (usually in the blastocyst stage) and the endometrium. In case of ectopic implantation, the endometrium may be replaced by other tissues.

Such a narrow definition is, however, not always used, which is unfortunate for the discussion of mechanisms involved. Much of the literature on "anti-implantation agents" is based on experiments in which, after treatment of female animals at any point during the preimplantation phase, uterine swellings (so-called "implantation sites") are counted at a relatively late stage, i.e., a number of days after initial contact formation between the trophoblast and the endometrium. Certainly, many complex tissue interactions and considerable remodeling processes are required until a mature placenta is established. It is usually impossible, in the described type of experiments, to decide which individual process was influenced by the drug treatment. Systematic morphologic investigations of all successive stages, combined with biochemical studies of compounds suggested to be related to implantation mechanisms, are rarely being carried out. This may be one reason why, although there is no lack of hypotheses on implantation mechanisms, concepts are highly controversial and incongruent, focusing either on regulation by ovarian (or trophoblast-derived) hormones, or on changes of cell surface characteristics, or on uterine motility, or decidualization, or vascular changes in the endometrium, including their regulation by prostaglandins and histamine, for example. According to the specialization of the investigator and depending on the type of

---

* To maintain the flavor of this original contribution, the editor has chosen not to rewrite the chapter in idiomatic English.
R.M.W.

experiment preferred and on the stage chosen for investigation, any of these aspects may appear to be of prominent importance for "implantation."

We would like to concentrate, in our discussion, on the *initial phase* of implantation, i.e., on mechanisms involved in the formation of the first intimate and specialized contact between trophoblast and uterine epithelium, with subsequent invasion. We will omit a discussion of other important aspects of implantation such as vascular reactions and decidual reaction, the hormonal regulation, the role of histamine and prostaglandins, topics that have been covered in a number of reviews.[9, 28, 36, 53, 69, 87, 108]

Recent experiments, using predominantly the rabbit and the mouse as models, suggest a central function for certain highly specific *proteinases,* in the initiation phase of implantation. They should be of interest to the gynecologist since *proteinase inhibitors* are being proposed and tried as additives to IUDs in order to reduce menstrual blood loss.[130, 131, 142] These inhibitors are in part identical with those found highly effective in interfering with implantation initiation, in the animal models.[25, 27] It seems necessary, therefore, to consider the possibility that proteinase inhibitors released from IUDs may have additional effects, i.e., interfere with implantation of the blastocyst, also in the human.

EXPERIMENTAL EVIDENCE FOR A SPECIFIC ROLE OF CERTAIN PROTEINASES IN IMPLANTATION INITIATION

It is a very old concept that degradation of maternal tissue components by factors released from the trophoblast may play an important role in implantation. In fact, in 1883, Graf von Spee,[126, 127] studying implantation in the guinea pig which is of the interstitial type as in the human, concluded on morphologic grounds that the trophoblast plays an active and cytolytic role, the endometrium showing signs of degeneration and disintegration. Although he apparently thought of an indirect influence by the embryo, suggesting a "biochemical process which is stimulated by the egg," his investigations initiated active search for degradative enzymes released from the trophoblast, particularly proteinases, during the following decades (discussed in Denker[25]). In fact, proteinases and proteinase inhibitors were found in both the trophoblast and the endometrium in the human shortly after the turn of the century. However, the simplifying concept that the invading trophoblast forces its way by digesting away components of the maternal tissues was later abandoned for being too mechanistic. It was nearly forgotten after the hormonal regulation of reproductive functions came into the center of interest.

In the light of recent experimental data, a role of hydrolytic enzymes in implantation must be reconsidered, although in a modified concept. Proteinases are certainly involved, but only part of their function, and possibly not even the physiologically most significant one, may be complete hydrolysis of maternal proteins to small peptides and amino acids as caused by *cathepsins.* Most members of this latter class have an acid pH optimum and are typical lysosomal enzymes. Some of them have been found to be in fact very active at implantation sites: Cathepsin B-type enzymes showing impressive activity in the cat,[30] the pig (Denker and Heap, unpublished) and the marsupial, the tammar wallaby;[35] a proteinase found in the guinea pig embryo may be related to it.[102]

Cathepsin D was found to show increased activity in total endometrial homogenates but decreased concentration in the uterine epithelium, at implantation sites of the rat.[93, 144]

Particularly interesting are, on the other hand, certain *alkaline or neutral proteinases which may act extracellularly* and for which experimental data suggest a special role in implantation initiation. According to biochemical studies, some of them may cause hydrolysis of only a few peptide bonds in their physiologic substrates, a process known as *limited proteolysis*. This interesting process can induce subtle but physiologically very significant changes in the substrate proteins, as demonstrated in a number of other systems: Certain highly specific proteinases are activators of proenzymes, some of them initiating complex chains of biochemical events involving mutual activation of various enzymes (examples: the blood clotting and the complement system) and liberating biologically active peptides (kallikrein-kinin system). When acting on cell surfaces, proteinases can elicit a great variety of cellular responses, e.g., changes in adhesive behavior, cell migration, secretion of proteins (e.g., fibronectin)[92] or of other enzymes, and they can cause metabolic changes or mitogenic activation;[54] (for additional references, see Denker[27]) and even trigger cell differentiation.[101] Enzymes which show these effects include highly specific proteinases like thrombin, causing limited proteolysis. Even the degradation of extracellular ground substance macromolecules, as part of the process of invasion, seems to involve primarily only partial degradation to high-molecular-weight products.[73, 83]

In fact, the implantation-associated proteinase which has been studied in most detail so far, blastolemmase, shows biochemical properties that are in accordance with such a concept, i.e., suggesting that the enzyme action is highly specific, cleaving only a limited number of peptide bounds in its physiologic substrates. The enzyme which can be extracted from the trophoblast of late preimplantation stage rabbit blastocysts exhibits not only a primary specificity for arginyl bonds (P_1) but also recognizes the adjacent amino acids in the neighboring positions P_2 and P_3, so that it can be expected to cleave only some of the arginyl bonds in a protein.[31]

Concerning the question about the cell biologic mode of action of proteinases in implantation initiation, most available information was obtained in experiments using the rabbit as a model, and some data have been obtained from the mouse. [25, 27, 28] The rabbit offers the advantage of having a greatly expanded blastocyst, around 5 mm in diameter at implantation, which facilitates analysis. In addition, due to its copulation-induced ovulation, developmental stages are defined precisely. It has, therefore, been chosen as the standard model in our laboratory, although it does show certain peculiarities mostly connected with its representing the central type of implantation. The problem posed by species differences will be discussed more in detail.

The most interesting proteinase operative in implantation initiation in the rabbit is the trophoblast-dependent *"blastolemmase.*"[21, 24, 25, 27, 31] With use of a gelatin substrate film test, the enzyme is found at the interface between trophoblast and uterine epithelium precisely during the phase of attachment of the abembryonic trophoblast, which is the beginning of implantation in this species. Noticeable blastolemmase activity is, therefore, demonstrable only for about one day, i.e., between 6 3/4 and 7 3/4 days post coitum (days p.c.).

Two major physiologic functions have been envisaged for blastolemmase: (1) in the process of dissolution of the so-called blastocyst coverings, and (2) in the attachment of the trophoblast to the uterine epithelium.

There is excellent experimental evidence for a central role of blastolemmase in the first-mentioned process, the *dissolution of the blastocyst coverings*. These are extracellular coatings composed principally of glycoproteins and equivalent to the *zona pellucida,* although in our model, the rabbit, they are of a more complex structure than, for example, in the human: when implantation is being initiated, they consist of 3 layers of different origin and composition (for more details, see below). For implantation, they need to be removed in order to bring the trophoblast into contact with the uterine epithelium. Blastolemmase is of central importance in this process, as shown impressively in experiments in which effective but *nontoxic proteinase inhibitors* (e.g., aprotinin = Trasylol®, antipain, soybean trypsin inhibitor, and others) were administered within the uterus in vivo (Denker[25] and unpublished results). The major effects seen were the same with all blastolemmase inhibitors although minor differences were noted.

Due to this treatment, dissolution of the blastocyst coverings does not take place, and they remain interposed between trophoblast and uterine epithelium so that formation of a cellular contact is impossible (Figs. 1A,B; 2). Therefore, blastocysts stay free in the uterine lumen.

An important detail of these experiments is the observation that the blastocysts remain viable for a number of days even though implantation was blocked. We like to put emphasis on this point because, as a rule, other investigations of "anti-implantation" agents have usually not ruled out the possibility that these drugs were perhaps simply causing degeneration of the embryos, which makes it impossible to derive any conclusions on specific cell biologic processes essential for implantation. In the experiments with inhibitors administered within the uterus the blastocysts continue to expand (although at a slightly reduced rate) in spite of their failure to implant. Electron micrographs have not given evidence for any major toxic damage to the trophoblast.[25] As a result of continued expansion, most of the blastocysts finally hatch mechanically from their coverings, although a proportion of them remains completely encased (particularly in experiments in which the inhibitor is being infused continuously into the uterine lumen). The fragments of the coverings typically remain sharp-edged, indicating absence of any lytic (blastolemmase) activity. In those blastocysts in which mechanical hatching occurs, the trophoblast can, of course, contact the uterine epithelium, although delayed (1–2 days). Interestingly, not all of the exposed parts of the trophoblast manage to attach under these conditions; the contact which is being formed mostly remains superficial (Fig. 2B) and invasion and erosion of maternal blood vessels are found only in few spots. Although most of the blastocysts in inhibitor-treated uteri are still nicely expanded around 2 1/2 days after the time when implantation has started in the controls (Fig. 3), they are finally being progressively resorbed.

In the initial series of these experiments, a single bolus of inhibitor had been injected into the uterine lumen of rabbits at 6 1/2 days post coitum, i.e., half a day before implantation initiation.[25] Since the intrauterine inhibitor concentration was found to decline rapidly, a more recent series used continuous infusion into the uterine lumen via Alzet® osmotic minipumps loaded with aprotinin (Trasylol®) and placed within the uterus at 5 1/2 days post coitum when midblastocysts are present.[90] Saline-loaded minipumps were used as controls placed in the contralateral uterus. In a sense, the minipumps serve as a model for medicated IUDs. The dimensions of the model used (Alzet® model No. 1701, now replaced by a larger model) easily allow intrauterine in-
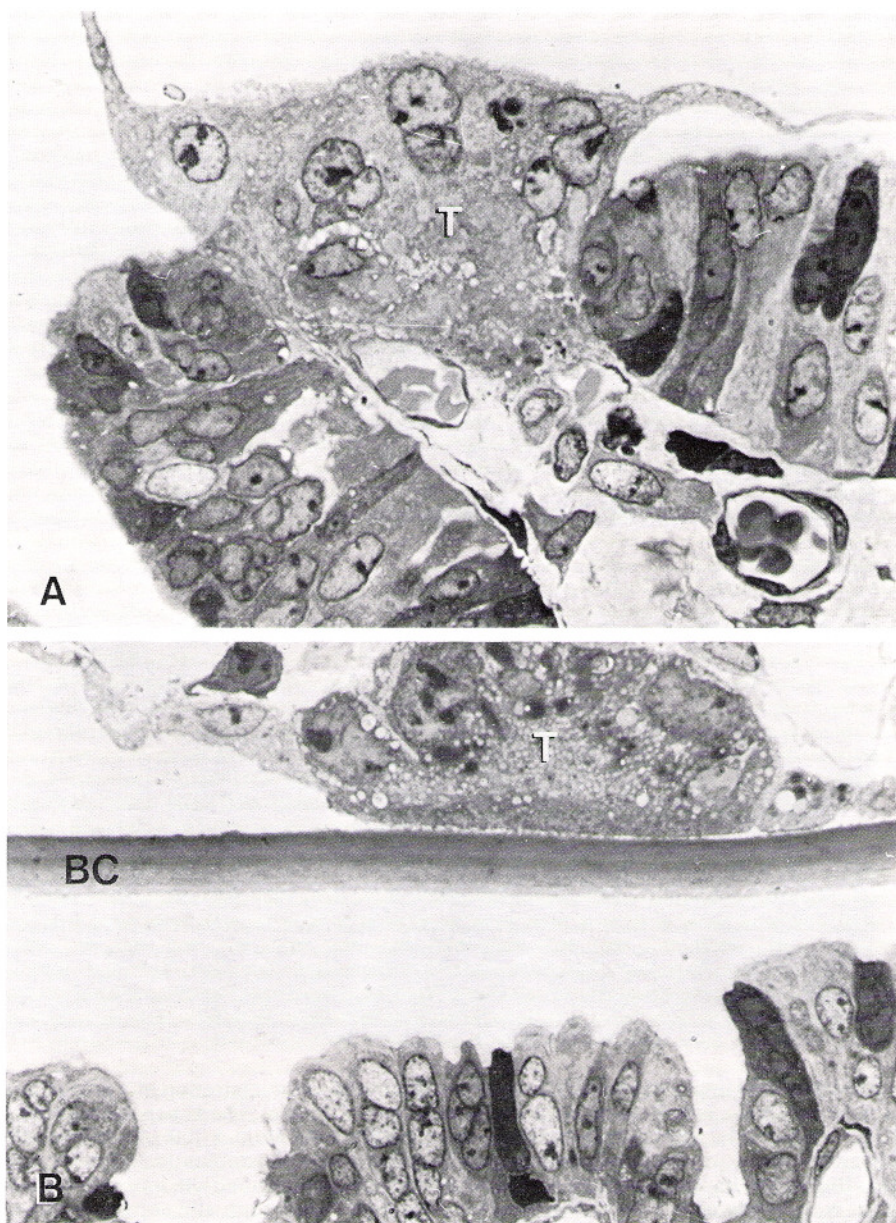
Fig. 1. Inhibition of implantation in the rabbit by intrauterine administration of a proteinase inhibitor. Stage shown is 7 1/2 days p.c. **A.** Control, Araldite section, toluidine blue stain. x 900. Antimesometrial endometrium and part of the abembryonic hemisphere of the blastocyst (above) are seen. A syncytial "trophoblastic knob" (T) has established contact with the endometrium, has invaded it and already reached the subepithelial capillaries. No remnants of blastocyst coverings are present anymore. **B.** As in **A,** except that 6 mg of aprotinin (Trasylol ®) had been injected into the uterine lumen one day before. Due to inhibition of blastolemmase activity, the extracellular blastocyst coverings (BC) have not been dissolved and remain interposed between the trophoblastic knob (T) and the uterine epithelium.
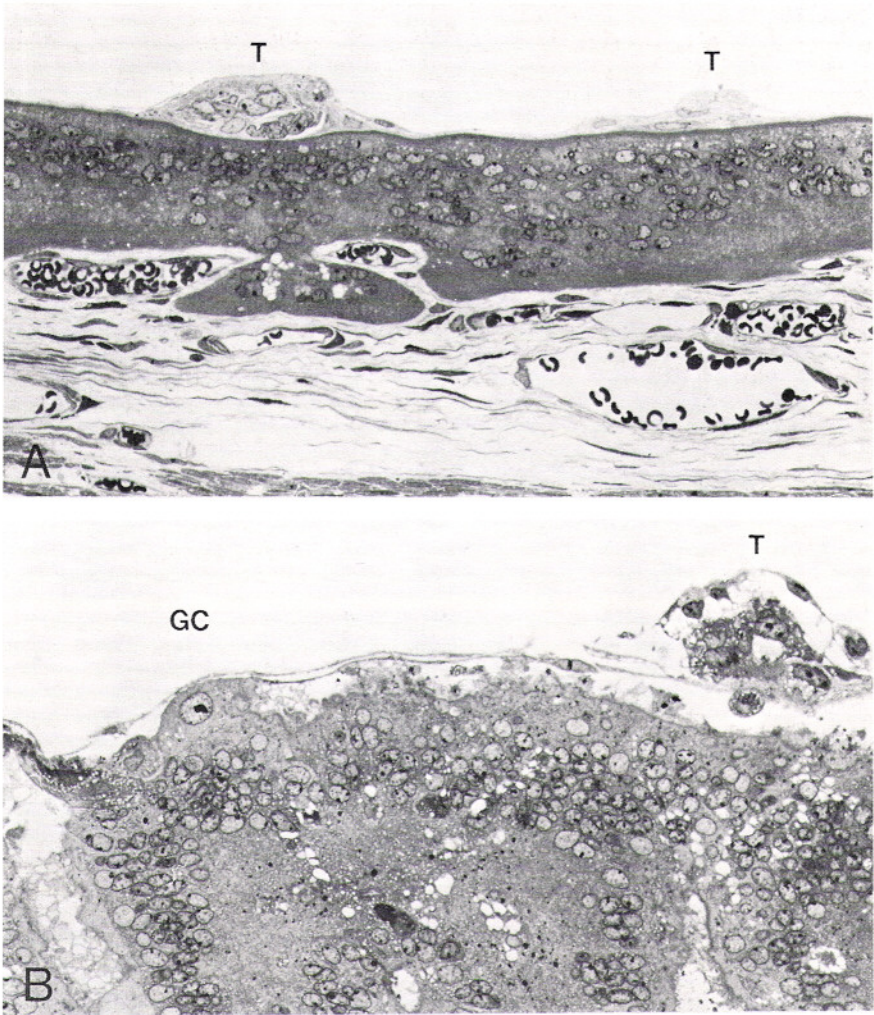
Fig. 2. Inhibition of implantation in the rabbit by intrauterine administration of a proteinase inhibitor. Stage shown is 8 1/2 days p.c. **A.** 6 mg of aprotinin (Trasylol®) had been injected into the uterine lumen at 6 1/2 days p.c. Araldite section, toluidine blue stain. x 350. In this segment of the abembryonic part of a blastocyst (above), two syncytial trophoblastic knobs (T) can be seen. Although in the controls they would have invaded the endometrium and a hemochorial contact would have been established by this stage, these knobs are still separated from the uterine epithelium (transformed into a broad symplasma) by the blastocyst coverings (dark line), which have been stretched due to continuing expansion of the blastocyst. **B.** As in **A,** another blastocyst from the same animal as shown there. In spite of inhibition of dissolution of the blastocyst coverings, many blastocysts finally manage to hatch mechanically due to their continuing expansion. At the site shown here, no remnants of blastocyst coverings are present anymore. Nevertheless, the trophoblastic knob (T) has not attached to the uterine epithelium and only a small portion of the cytotrophoblast has. Here, a trophoblastic giant cell (GC) has formed. There is no sign of deep invasion.

Fig. 3. The two uteri of a rabbit excised at 9 1/2 days p.c. after 12 mg of aprotinin (Trasylol ®) had been injected into the lumen of the left uterus at 6 1/2 days p.c. The right uterus had received the same amount of vehicle fluid as a control. Since the proteinase inhibitor does not cause immediate degeneration of the blastocysts but only interferes with the initiation of their implantation, the two blastocysts at the left side have not degenerated yet but have continued to expand, although at a slower rate than the five conceptuses at the control side (right). (No. of corpora lutea: left side 2, right side 6).

sertion in the rabbit, and, as the controls reveal, there is only a minor nonspecific mechanical IUD effect (leukocytosis, although variable, unusually remaining slight, and the nonspecific antifertility effect remaining negligible) because this pump occupies only a small part of the length of the uterus, and it distends the uterus only slightly. (Fertility depressing effects of nonmedicated IUDs have been found to be restricted to the endometrial area in contact with the device.)[81] The results of the experiments observed at various stages between 7 1/2 and 11 1/2 days p.c. confirm the main results obtained with single intrauterine injections of the inhibitor: Dissolution of the blastocyst coverings is strongly inhibited whereas the blastocysts survive and continue to expand. Degeneration of the embryoblast is seen more rarely, after continuous infusion of the inhibitor than after a single intrauterine bolus injection. In fact, some embryos were found, in the minipump experiments, beautifully differentiated showing neural tube formation and somites, in spite of the fact that they were still completely encased in their blastocyst coverings, i.e., they were totally nonimplanted and free in the uterine lumen. Such a condition had, to our knowledge, never been seen before in a eutherian mammal except for the horse (Betteridge, personal communication) but was known from marsupials (see, e.g., Renfree[110]).

About 25 percent of the embryos remained completely encased in the coverings,

after continuous intrauterine aprotinin infusion, whereas the others managed to hatch mechanically. It seemed interesting to note whether the trophoblast would be able to *attach* and to *invade* into the endometrium, in spite of the presence of the proteinase inhibitor. Some of the exposed trophoblastic knobs of the abembryonic hemisphere were indeed found to invade and to erode maternal blood vessels. Many of them did not, however, even if no remnants of blastocyst coverings were interposed between them and the endometrium any longer. This does not necessarily prove that aprotinin-sensitive proteinases are involved in attachment and invasion; it can, alternatively, be explained using a concept proposed by Böving,[8] i.e., that there is only a small time window for invasion of trophoblastic knobs into the endometrium, the end of the susceptibility of the endometrium being determined by the time when uterine epithelial cells fuse and form a broad symplasma after 7–7 1/2 days p.c. After that time, directional invasion of trophoblastic knobs aiming at subepithelial blood vessels may be impossible (because there is no longer any channeled transport of metabolites). In fact, fusion of trophoblast with the uterine epithelium seems basically to be possible, in the inhibitor-treated uteri, but deep invasion is rare in the abembryonic-antimesometrial region.

Implantation consists of two different phases in the rabbit. The phenomena discussed above are part of the first phase of implantation, which takes place in the abembryonic region of the blastocyst, normally oriented towards the antimesometrial part of the endometrium (Fig. 4). This process results in the formation of a yolk sac placenta, which is essentially an ephemeral organ. About 1 1/2 days after the beginning of abembryonic implantation, fusion of the trophoblast with the uterine epithelium also starts at the embryonic pole of the blastocyst, adjacent to the mesometrial part of the endometrium. This leads to the formation of the definitive chorioallantoic placenta (again hemochorial). After infusion of aprotinin into the uterus with the minipump, the latter process did *not* seem to be impaired at all in those blastocysts that had managed to hatch mechanically from their coverings, if no remnants of the latter remained interposed.[90] This seems to indicate that adhesion of the trophoblast to the uterine epithelium and invasion may not be dependent on aprotinin-sensitive proteinases like blastolemmase. The physiologic function of the latter then would seem to be restricted to the dissolution of the blastocyst coverings, comparable to a *hatching enzyme*. However it cannot be excluded at present that higher doses of the inhibitor would perhaps interfere with invasion.

The combined morphologic and biochemical evidence from investigation of normal implantation sites and of inhibitor-treated uteri suggests that all major effects of the mentioned proteinase inhibitors are due to direct inhibition of blastolemmase.[25] However, indirect effects on implantation would be possible if inhibitors (like antipain) interact with proteinases involved in hormone-dependent changes in endometrial cell physiology, as proposed for the mouse and rat.[67, 105]

Is there any evidence for a role of a blastolemmase-like enzyme in implantation in *other species?* Our knowledge of the physiologic way of *zona-shedding* in other species is, unfortunately, very limited. We do not know anything about the situation in the human, not even the time at which shedding occurs here, because the available specimens of the critical period are rare and have not been preserved in a way appropriate to retain the zona. In the *rhesus monkey* (which, however, shows a number of differences from the human, such as endometrial plaque reaction, central type of im-
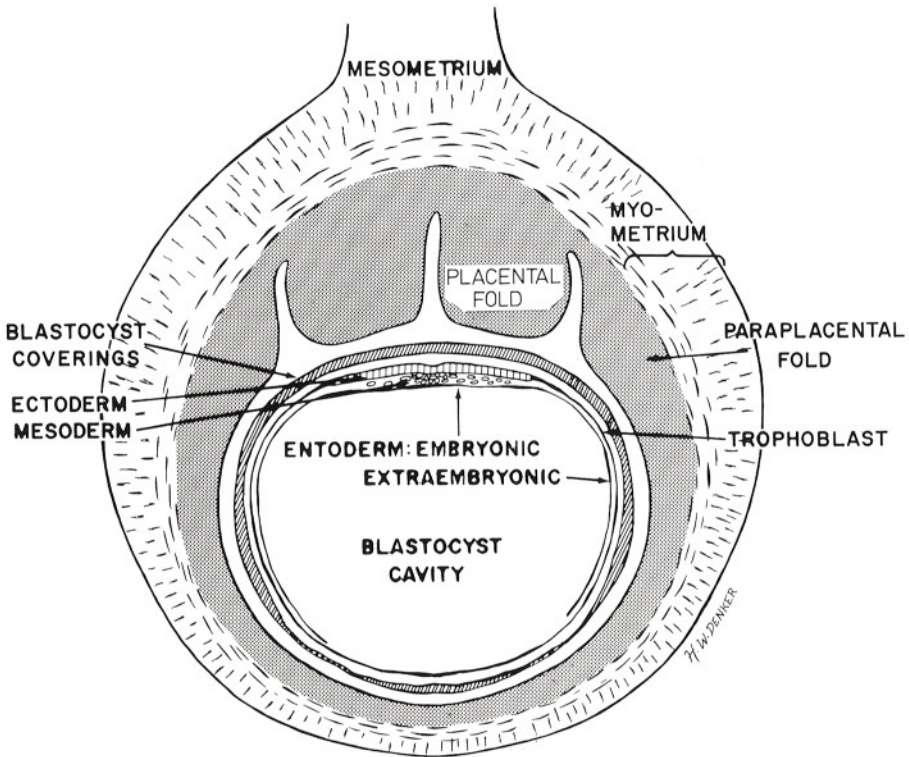
Fig. 4. Schematic sketch of the topographical relationships between blastocyst and uterus at the time of implantation initiation in the rabbit, i.e., 7 days p.c. Cross-section through the uterus. The embryonic disc is oriented towards the mesometrial side of the uterus, the abembryonic pole faces the antimesometrial endometrium. The blastocyst is still completely encased in its extracellular coverings, although the dissolution of the latter is just starting in the abembryonic-antimesometrial region where implantation begins.

plantation, late onset of implantation), empty zonae flushed from the uterus together with zona-free blastocysts seemed to indicate that here the embryos may hatch mechanically, perhaps a day or so before implantation.[45] Nothing is known about any proteinases of the uterine secretion or of the trophoblast which might be involved. In the *cat,* there is morphologic evidence that the zona pellucida is dissolved under the influence of factors depending on the abembryonic and lateral trophoblast (corresponding to the future girdle-shaped invasion zone?),[30] but the gelatin film failed to identify a specific proteinase possibly responsible, other than the general cathepsin B-like activity of the trophoblast. In the *mouse,* blastocysts can easily hatch by rupturing the zona, in vitro. However, histologic investigation of implantation sites reveals that the normal process is by lysis.[88] It has been proposed that a uterine secretion-derived enzyme assumed to be identical with a caseinolytic proteinase showing the same stage- and hormone-dependent changes of activity is responsible for this as well as for changing cell surface properties so that attachment of the trophoblast can occur ("implantation-initiating factor,"[91, 106]). No direct experimental evidence is available for the latter

assumption, however, while evidence for a role in zona lysis is good. It is possible that the factor described by Mintz[91] is identical with a chymotrypsin-like enzyme described recently in mouse uterine flushings,[59, 60] showing the same dependence on an ''estrogen surge'' (superimposed on progesterone action). (For comparison: rabbit blastolemmase is progesterone-dependent, see Denker[23]. There is no evidence for a physiologically significant estrogen surge in the rabbit or in the human.) The physiologic function for another estrogen-dependent uterine secretion proteinase described in the rat (and mouse), possibly being more close to elastase, remains to be defined, since it was found capable of dissolving the zona of unfertilized but not of fertilized eggs.[113, 114]

For a number of other proteinases described in the human and in animals, it remains unclear whether they are at all involved in the process of implantation, because they were studied either in uterine tissues remote from implantation sites, or in trophoblast of stages far beyond those discussed here.[62, 68, 96, 100, 134, 145]

Investigations of *plasminogen activator* activity present in mouse trophoblast during the phase of *stromal invasion*[128] have received interest because the same enzyme was found to be secreted in considerable amounts by many (although not all) highly invasive tumor cells and was proposed to be of major importance for tumor invasion. Using the macrophage as a model, evidence was presented suggesting that plasminogen activator may be an integral part of the whole proteinase system involved in degradation of connective tissue ground substance.[139, 140] As discussed earlier,[25, 27] experimental evidence for a major role in the initiation phase of implantation is lacking; in fact plasminogen activator activity of blastocysts and of endometrial and uterine fluid decreases towards implantation and is low at early implantation sites (see also Denker, unpublished).[11, 82, 117, 130, 137] The plasminogen activator found in cleavage stage embryos may have been adsorbed at the zona and carried from the ovary; its function is unknown.[121] ε-Aminocaproic acid, an inhibitor of plasminogen activation that does not affect blastolemmase noticeably, does not interfere with implantation when administered intrauterally in the same way as described for aprotinin for example.[3, 25] Likewise after oral administration of ε-aminocaproic acid, the implantation rate was not changed significantly and even tended to be slightly increased.[3] Increased fibrinolytic activity may indeed be an unfavorable condition for implantation because it is found in endometria adjacent to various types of IUDs (apparently not including progesterone-releasing IUDs)[11, 50, 78, 79] although in the uterine secretion, the activity remains unchanged or is reduced due to the presence of inhibitors.[11, 130]

The physiologic function of trypsin- and chymotrypsin-like enzymes described as occurring in mouse *blastocysts* is still unknown.[17] Proteinase inhibitors including aprotinin have been applied within the uterus also in the mouse using a slow release device,[16] and an antifertility effect was noted when ''implantation sites'' were counted at a late postimplantation stage. Unfortunately, no morphologic investigation of periimplantation stages was performed, so that these investigations do not provide any arguments for our discussion whether the major role of proteinases is in zona lysis, in changing cell surface characteristics for adhesion, or in other physiologic processes. Recently, attachment of the mouse blastocyst to mouse uterine, embryo, or L cell monolayers in vitro was described to be inhibited by soybean trypsin inhibitor (which gives basically the same results as aprotinin in the rabbit in vivo system [Denker, unpublished]), and to be stimulated by trypsin.[74] In a subsequent series of similar in vitro

experiments using mouse decidual cell monolayers, it was found that soybean trypsin inhibitor interfered particularly with attachment of the blastocyst (indicating the need for a trypsin- or chymotrypsin-like enzyme), while *p*-nitrophenyl–*p´*-guanidinobenzoate (NPGB) inhibited both attachment and outgrowth (interpreted as indicating a need for plasminogen activator activity in addition to a trypsin-like enzyme).[75] ϵ-Aminocaproic acid had little effect, which is in agreement with our rabbit experiments in vivo.[25] Interpretation of experiments with NPGB seems problematic, however, since it was shown to have rather toxic side-effects in the rabbit in vivo system.[25]

In another in vitro model system, digestion of films of extracellular matrix by mouse trophoblast outgrowths is being studied.[141] Early spreading of trophoblast onto the substrate was found to depend upon the presence of plasminogen, but subsequent invasion and matrix digestion were plasminogen-independent. Characterization of the proteinase(s) involved in the latter process has not been achieved thus far. Unfortunately, this fascinating trophoblastic outgrowth system suffers from some of the drawbacks of all two-dimensional culture systems.[86] There is some doubt whether cell polarity is still maintained in the outgrowth, since morphology suggests that the side of the trophoblast that attaches to the substrate may finally represent the basal portion of the cell rather than its apex (if any such polarity can be attributed to it).

It is possible, therefore, that in addition to playing a crucial role in dissolution of blastocyst coverings (zona pellucida), certain proteinases of the trypsin/chymotrypsin family are involved in attachment of the trophoblast to the uterine epithelium and invasion. Since our in vivo systems failed to give clear-cut evidence in favor of this, we prefer to withhold any firm statements until we can perform further experiments, using preferably the in vivo system and applying inhibitors that were first tested in vitro against the various proteinases being isolated from trophoblast, endometrium and uterine secretion.

THE PHYSIOLOGIC REGULATION OF IMPLANTATION INITIATION

We will concentrate here on the cell biologic mechanisms of contact formation and refrain from discussing the hormonal regulation. A word of caution should be adequate, however, in this context: Most investigations on the hormonal regulation have used the rat and mouse as a model, pointing out the importance of a preimplantation rise in estrogen secretion. However there is no evidence for a physiologic function of elevated maternal estrogens in initiation of implantation in most other species (including man), and it may be a special feature of such species that are able to undergo delay of implantation (embryonic diapause).[89] It is possible that the same applies to the interesting factor found in uterine fluid of mice, which inhibits uridine incorporation and which may regulate diapause,[138] although it was claimed that comparable inhibiting factors do exist also in a species without diapause—the pig.[49]

If proteinases play a role in the initiation of implantation, as suggested by the experiments discussed above, *proteinase inhibitors* can be expected to be involved in regulating this process. Proteinase inhibitors are present in all tissues where proteinases are being produced or serve their physiologic function, as in the pancreas (digestive pro-

teinases and, e.g., pancreatic secretory trypsin inhibitor), mast cells (mast cell pro-teinases and, in the cattle, aprotinin) or in the blood plasma (blood clotting cascade proteinases and their inhibitors). Proteinase inhibitors have been proposed to mediate, at least in part, the resistance of various tissues to invasion.[76, 136] It was assumed, therefore, that proteinase inhibitors ought to be present also in the uterus and the trophoblast, and it was possible to prove their existence experimentally (for references see [25]; for more recent data see [7, 11, 18, 47, 56, 95, 118, 130]). Although it had been proposed that uteroglobin, the dominant uterine secretion protein of the rabbit in the preimplantation phase, is a proteinases inhibitor,[5, 65] it was thought that this might be due to a low molecular weight inhibitor co-purifying in the same fraction.[26] In fact, separation from uteroglobin and characterization as a very specific trypsin inhibitor has recently been achieved.[56] Plasma proteinase inhibitors shown to be present in human uterine fluid[11, 130] were found to be able to inhibit rabbit blastolemmase (used as a model as long as cor-responding human enzymes are unknown).[26]

Uterine proteinase inhibitor activities are sensitive to maternal steroid hormone levels.[4, 11, 47, 56, 95, 130] The low molecular weight trypsin inhibitor of rabbit uterine fluid was found to show a pronounced peak of activity in the preimplantation phase but to decline towards implantation.[56] Regulation of proteinase inhibitor activity levels in the uterine lumen may be one physiologic way of regulating implantation initiation. Such a concept fits nicely the results of experiments on the interference with implantation in-itiation by intrauterine administration of exogenous proteinase inhibitors in vivo, as discussed earlier. For the physiologic situation, we would predict that, in addition to regulation of inhibitor levels by maternal steroid hormones, local effects exerted by the blastocyst might also play a role perhaps in a way comparable to phenomena described for endometrial amino acid arylamidase activity:[25, 135] the blastocyst locally stimulates discharge of arylamidase from surrounding uterine epithelium in the preimplantation phase and causes exhaustion of it at the implantation site.

In preliminary experiments, we did not find any evidence that the low molecular weight inhibitor isolated from rabbit uterine secretion inhibits blastocyst proteinase (as determined with chromogenic substrates), although this had been expected since the en-zyme interacts with typical trypsin inhibitors. Therefore, the inhibitor may be directed against the trypsin-like enzyme found in uterine fluid.[21, 25, 34, 70, 71] The simplistic model that the uterus controls directly, via its inhibitor, the blastocyst proteinase and thereby implantation may not apply.

With regard to the special features of animal models representing the central type of implantation, a word of caution may be appropriate concerning their usefulness when it is intended to extrapolate conclusions to species like the human with interstitial implantation. A typical feature of species with central implantation is to exhibit a con-siderable degree of blastocyst expansion. As a consequence, a large volume of fluid ac-cumulates in the blastocyst cavity, and surface area increases markedly to permit exten-sive metabolic exchange. Two consequences can be derived from this. For nutrition of the large blastocyst, considerable amounts of special secretory proteins are being pro-duced by the endometrium, some of them perhaps serving carrier functions as discussed for pig uteroferrin[111] or rabbit uteroglobin.[5] On the other hand, effective "buffer" mechanisms are required in order to avoid uncontrolled spreading of biochemical reac-tions (in the sense of activation of enzymes) or uncontrolled diffusion of metabolites or

mediator molecules in such a large uterine cavity. Many of the proteins present can, therefore, be expected to be (a) typical enzyme inhibitors or (b) molecules with relatively weak binding properties for various compounds. The quantity of such molecules will be much greater in these species than in those with interstitial implantation, and hormone-controlled changes in secretory patterns will be much more pronounced in the former than can be expected to be the case in the human. Those animal models are very useful for studies of the mechanisms of hormone action in general, but when trying to apply the concepts derived we must be very cautious and keep in mind that the secretory phenomena are at least greatly exaggerated, in these animals. It is even possible that certain factors playing an important role in the mentioned species, during the late preimplantation phase, may not be needed at all in the human.

Regulation of *secretion* of the enzyme (and proenzyme?) itself would, of course, be another way of steering the process of implantation. Production of *blastolemmase* activity depends on the presence of (abembryonic) *trophoblast* tissue, as shown in a number of experiments.[24, 33] The enzyme can be extracted from trophoblast of the appropriate stage.[31, 34] However, experiments demonstrating unequivocally that the trophoblast is the site of synthesis of the enzyme molecule are still lacking. Uptake from the uterine secretion cannot be excluded at present, and nothing is known about possible proenzyme activation processes. Proteinases are also present in rabbit *uterine secretion*[21, 25, 70, 71, 119] and seem to fall into the classes of tryspin-like and chymotrypsin-like enzymes.[25, 34] It may be expected that production of uterine secretion proteinases may be regulated by maternal steroid hormones, but besides the dependence on an estrogen surge in the mouse and rat mentioned above little is known about this at present. There is some evidence that the blastocyst stimulates locally the secretory process or the release of cell surface-bound enzymes of the adjacent uterine epithelium.[25, 85, 97, 135] Trophoblast-derived and uterine secretion-derived proteinases probably act together in a concerted way to cause the dissolution of the blastocyst coverings (and attachment?).

It appears possible that enzymes like blastolemmase may be involved in bringing about the correct *orientation of the blastocyst* in the uterus at implantation initiation. In the rabbit model, the normal orientation is with the embryonic disc facing the mesometrial endometrium and the abembryonic pole of the blastocyst facing the antimesometrial part of the endometrium (Fig. 4). It was proposed by Böving[8] that a pH gradient between the mesometrial and the antimesometrial part of the uterine epithelial surface and a potential of the abembryonic pole of the blastocyst to develop a rise in pH (due to bicarbonate production, transport and CO_2 release into the maternal circulation) act together in this respect. It was proposed that the result is an increase in adhesiveness of the blastocyst coverings at the abembryonic pole, this pole becoming trapped in the antimesometrial niche of the endometrium, due to increased pH. In fact, measurements with pH microelectrodes in vivo give values of 7.32 and 7.54 for the mesometrial and the antimesometrial surface of the endometrium, respectively, at this stage (Fig. 5).[104] Comparison with the pH profile of blastolemmase activity (Fig. 6) reveals that the higher pH would increase enzyme activity. The blastocyst at first rotates freely in the uterine lumen. After blastolemmase production (which appears to be dependent on the abembryonic trophoblast)[24] has started, a positive feedback mechanism can be expected to come into effect as soon as the abembryonic pole meets the antimesometrial endometrium with the higher pH value. Limited proteolysis of the blastocyst coverings by
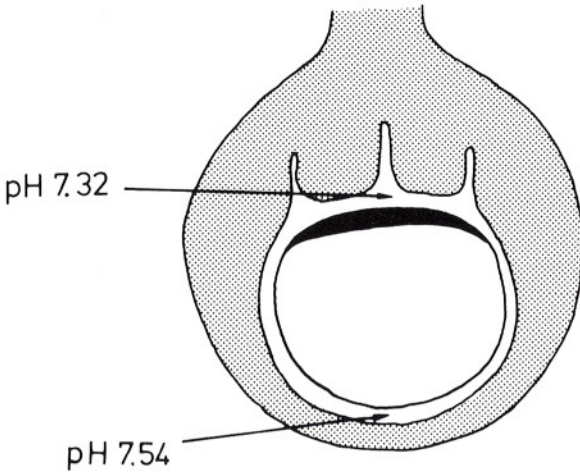
Fig. 5. The pH values measured at the endometrial surface in vivo using microelectrodes are markedly different at the meso-metrial and the antimesome-trial side of the rabbit uterus (7 days p.c., values from [104]). The blastolemmase-dependent dis-solution of the blastocyst cov-erings starts normally in the abembryonic-antimesometrial region.
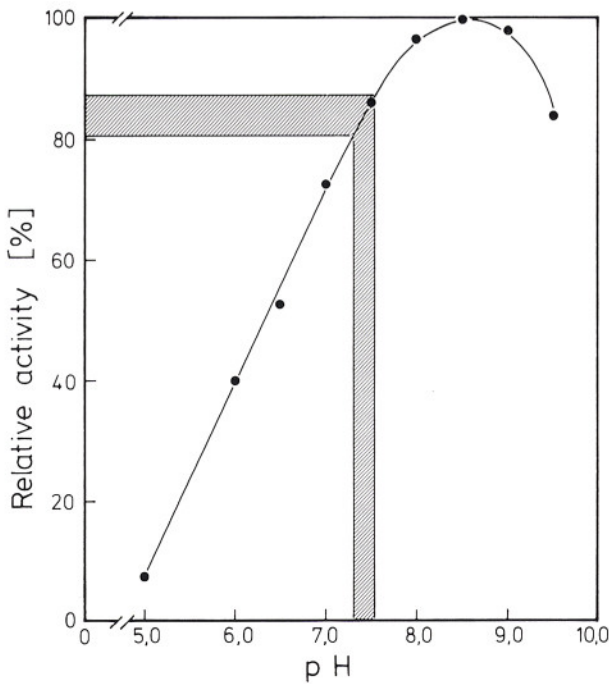
pH 7.32

pH 7.54

Fig. 6. The pH profile of rabbit blastocyst proteinase (blastolemmase) as measured with TosGlyProArg-*p*-nitroanilide as substrate.[31] The difference in pH values measured between the mesometrial and the antimesometrial aspect of the endometrium (Fig. 5) is indicated (hatched area). A pH increase in this range can be expected to cause a marked increase in enzyme activ-ity. This is proposed to be one mechanism involved in bringing about the correct orientation of the blastocyst in the uterus, with its abembryonic pole (higher blastolemmase activity) facing the antimesometrial endometrium (higher pH value).

blastolemmase will make them soft and adhesive, and may help to anchor the blastocyst in this, the correct, orientation. Manipulation of the uterus during this sensitive phase (as with proteinase inhibitor, or saline, injection; any fibrin deposition in the uterine lumen) was found to increase the incidence of dystopic orientation and implantation in the uterus.[25]

Thus, the concept of a physiologic role of pH gradients in the uterus for implantation, and the concepts of the function of proteinases like blastolemmase in implantation initiation, can easily be reconciled. The same concepts can be applied, hypothetically, to species in which the lysis of blastocyst coverings (zona pellucida) occurs long before attachment of the trophoblast: Here, changes of adhesiveness of glycoproteins may occur at the cell surfaces rather than at the zona but may obey comparable laws.

THE ROLE OF FACTORS OTHER THAN PROTEINASES IN THE EARLY PHASES OF IMPLANTATION

For completeness, we need to bring into the picture additional factors that are probably involved but for which experimental evidence is still very poor. As we have seen in the experiments with intrauterine proteinase inhibitor administration, a role of proteinases in cell contact formation itself remains questionable in spite of effects reported for in vitro systems. Even if proteinases change cell surface properties in such a way that adhesion is promoted, this does not explain the type of interaction involved in the ensuing binding.

Individual uterine luminal proteins seem to be recognized and bound differentially by rat blastocysts[133] but the mechanism is unknown. For many years, attention has been directed at the possibility that *carbohydrate side chains of cell surface glycoproteins* may be involved in mediating adhesiveness between trophoblast and uterine epithelium. Decrease as well as increase in density of certain carbohydrate groups as well as of negative charge (due to bound sialic acids or sulfate ester groups) have been reported to occur at the cell surface.[19, 20, 43, 44, 46, 57, 64] There are conflicting results about the effects of inhibition of glycoprotein synthesis by tunicamycin, however.[123, 129] It was reported that removal of cell surface-bound sialic acids by neuraminidase [0.2 ml (!) injected into the uterus, or flushed blastocysts treated in vitro and retransferred] is an effective means of interfering with implantation in mice.[107] Histochemical investigation however failed to show a topographical correlation of neuraminidase activity with the attachment sites in the rabbit,[22] and neuraminidase had no effect on the attachment of mouse blastocysts to glass surfaces in vitro.[52] As far as the type of interaction is concerned, consideration of solely the cell surface *charge* situation may be thought to be too simplistic. The Roseman hypothesis[112] involving cell surface-bound *glycosyl transferases* and enzyme-substrate binding, although attractive, thus far lacks experimental support in this system.

Another type of recognition mechanism would involve *lectin-like molecules,* e.g., present at the surface of one of the two partners and binding to certain carbohydrate side chains of glycoproteins found at the surface of the other one. *Binding sites* for exogenous lectins, i.e., carbohydrate groups, are being identified at the trophoblast and the uterine epithelial surfaces, some of them showing stage-dependent changes and differential distribution at the embryonic versus the abembryonic pole of the blastocyst ([10, 13, 44, 55, 84,]

[99, 146, 147]; Nalbach and Denker, in preparation). However, knowledge is still limited and some of the data concerning decrease or increase towards implantation are still controversial.[10, 44, 147] In particular, there is no proof for the presence of lectin-like molecules at these sites, and experimental evidence for an interference with implantation by administration of the appropriate competitive sugars is lacking. Intrauterine injection of the lectin, concanavalin A, was reported to block implantation in the mouse;[58] however, we observed in preliminary experiments performed in the rabbit that this lectin can cause degeneration of the blastocysts so that additional experiments are needed in order to clarify the mode of action (Denker and Nalbach, in preparation). Since several laboratories are working on this complex of questions, we may expect more data to become available during the next few years.
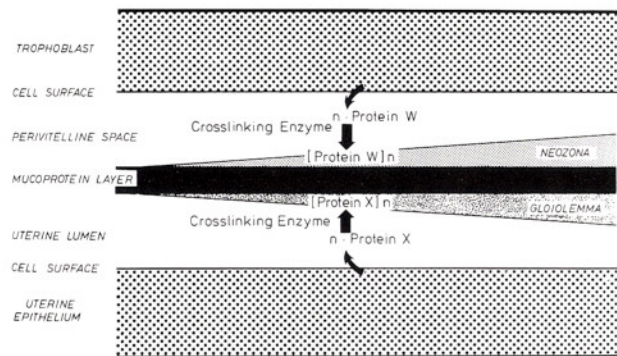
In any case, the initial contact that may still be weak and may be established by the mentioned mechanisms will have to be consolidated during the subsequent phase of firm attachment and invasion during which even intercellular junctions are being formed between trophoblast and uterine epithelium.[115] We are at present testing the hypothesis that cross-linking enzymes may be involved. In fact, there is a considerable *polymerizing/cross-linking capacity* in the uterus-blastocyst system in our model, the rabbit, as shown impressively by the continuous deposition of extracellar material, the *blastocyst coverings,* during the preimplantation period (Fig. 7A). These blastocyst coverings are in a sense equivalent to the zona pellucida. In the rabbit, the mucoprotein layer, derived from tubal secretion, is being deposited around the egg, i.e., outside the zona pellucida, during tubal passage; the zona is being dissolved shortly after entering the uterus (i.e., around 3 days before implantation) and is being replaced by a new layer formed at the inside of the persisting mucoprotein layer, the *neozona* (Fig. 7A).[32, 51] The neozona appears to be formed partly from secretory products provided by the trophoblast, this being a new aspect of the physiology of preimplantation stage trophoblast. However, not only trophoblast-derived factors but also certain components of the uterine secretion appear to be essential.[48] In addition, the mucoprotein layer is also being reinforced by uterine secretion material deposited at the outside of the blastocyst coverings, the so-called *gloiolemma.*[8]

The continuing deposition of extracellular material around the blastocyst coverings is not unique for the rabbit and not as bizarre as it may appear at first glance. Deposition of an additional layer at the inside of the zona pellucida was shown in the fur seal (so-called subzonal layer)[39] and the horse.[6] All these species have in common that they represent the central type of implantation, i.e., the blastocyst attaining a large size, staying in a topographical sense in the (previous) uterine lumen although the trophoblast may finally erode the endometrium and form a hemochorial (rabbit) or endotheliochorial (carnivores) contact. In many species of this group, no additional layers of blastocyst coverings become morphologically discernible, although the fact that the thickness of what appears to be zona pellucida remains unchanged in spite of considerable expansion of the blastocyst, probably indicating that material is being added (from the inside or the outside) without a stratification becoming apparent (cat, see[30]). On the other hand, there is some indication that the addition of material to the zona may be an even more widespread phenomenon including species in which the blastocyst does not expand considerably, therefore possibly also including the human (for more data see[25]).
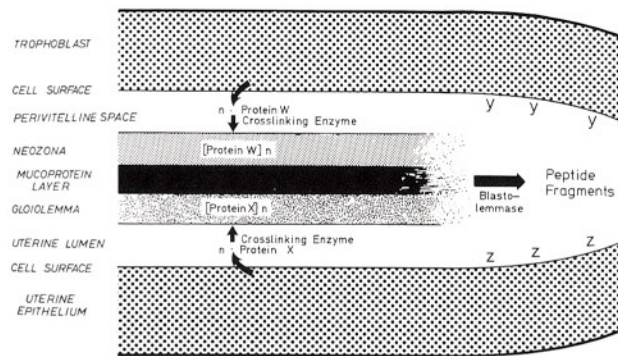
The *mechanism of deposition of this extracellular material* is still unknown. In formation of fertilization membranes, as studied in sea urchins and amphibia, it may involve precipitation of glycoproteins by lectins, perhaps ion-dependent, or cross-linking by enzymes like ovoperoxidase [116, 120] as well as further processing by limited proteolysis. [12, 143] Nothing is known about a comparable role of a peroxidase in the blastocyst-uterus system, although an estrogen-dependent endometrial peroxidase is well known and has been used extensively in studies of hormone action. [2, 37, 66, 72] We have preliminary evidence suggesting that transglutaminase (coagulation factor XIIIa) (forming isopeptide cross-links between glutamic acid and lysine) or a related enzyme may be involved in neozona formation. It has recently been suggested [94] that transglutaminase, which was found to be highly active in the uterus and to show an increase in activity during early pregnancy (although it was not indicated whether this was determined in the endometrium or the myometrium), [1, 14] is involved in masking embryonic antigens at the trophoblast surface by cross-linking them with uteroglobin, the dominant uterine secretion protein during the preimplantation phase in the rabbit (although not found unequivocally in other species). We are testing the alternative hypothesis that a transglutaminase-like enzyme is involved in cross-linking extracellular material as part of the processes of (1) formation of the neozona (and the gloiolemma?) and (2) establishment of firm adhesion of the trophoblast to the uterine epithelium. In fact, the polymerizing potential continues to be present in the rabbit uterus-blastocyst system, during the implantation phase. Normally, all layers of blastocyst coverings are being dissolved under the influence of blastolemmase at implantation initiation (Fig. 7B), uterine secretion enzymes probably contributing to the process so that peptide fragments are finally formed. If blastolemmase is inhibited experimentally, lysis of the coverings is not only prevented but deposition of both neozona and gloiolemma continues, [25, 51] showing the presence of the polymerizing factors.

There is some evidence for a role of transglutaminase in cell adhesion in other systems. [15, 80] Blastolemmase is highly selective to substrates; therefore, after dissolution of blastocyst coverings, (glyco)-proteins may remain at the trophoblast and endometrial surfaces, which are blastolemmase-resistant and which can mediate attachment, at first perhaps by a lectin receptor-like mechanism and then by being cross-linked by an enzyme like transglutaminase. We have immunohistochemical evidence that a fibrinogen-like antigen is present at the uterine epithelial surface (not the trophoblast). We know that fibrin is blastolemmase-resistant [25] and these molecules may, therefore, mediate attachment. After proteinase inhibitor injection into the uterine lumen, fibrin deposits form more often (and cannot be dissolved because of low fibrinolytic activity at implantation sites, or because of direct plasmin inhibition); blastocysts attach preferentially to these clots, often maloriented with respect to the mesometrial-antimesometrial axis.
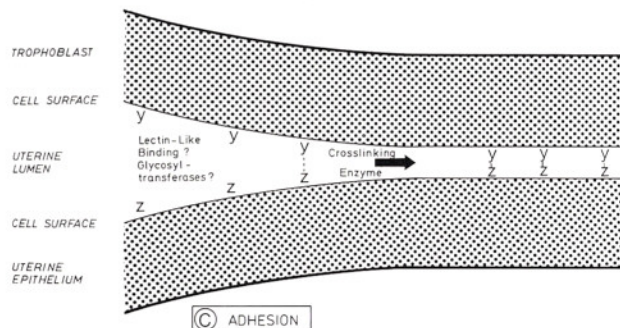
The molecular mechanisms involved in the phase of implantation that follows adhesion, i.e., *epithelial penetration,* may again involve the action of certain proteinases, as suggested by investigations done in other systems, showing that proteinases may regulate the degree of cell adhesion. [132] We do not know, however, whether these postulated proteinases, if they exist, are the same as discussed for the initiation phase of implantation or perhaps a different system. In experiments using the so-called trophoblastic outgrowth system in vitro [63] as a model, attachment to the culture dish or to cell monolayers, and outgrowth (the latter being used as a model for invasion) were

TROPHOBLAST

CELL SURFACE

PERIVITELLINE SPACE — Crosslinking Enzyme — n · Protein W

[Protein W] n — NEOZONA

MUCOPROTEIN LAYER

[Protein X] n — GLOIOLEMMA

Crosslinking Enzyme

UTERINE LUMEN — n · Protein X

CELL SURFACE

UTERINE EPITHELIUM

PREIMPLANTATION PHASE:
(A) FORMATION of NEOZONA and GLOIOLEMMA

TROPHOBLAST

CELL SURFACE

PERIVITELLINE SPACE — n · Protein W — Crosslinking Enzyme — y y y

NEOZONA — [Protein W] n

MUCOPROTEIN LAYER — Peptide Fragments

GLOIOLEMMA — [Protein X] n — Blasto-lemmase

UTERINE LUMEN — Crosslinking Enzyme — n · Protein X

CELL SURFACE — z z z

UTERINE EPITHELIUM

IMPLANTATION INITIATION:
(B) DISSOLUTION of BLASTOCYST COVERINGS

TROPHOBLAST

CELL SURFACE — y

UTERINE LUMEN — Lectin-Like Binding? Glycosyl-transferases? — y — Crosslinking Enzyme — y y y

z — z z z z

CELL SURFACE — z

UTERINE EPITHELIUM

(C) ADHESION

Fig. 7. Simplified diagram to illustrate some major molecular events shown or proposed to be involved in implantation initiation. In the *preimplantation phase* (**A**), deposition of extracellular material at the interface between trophoblast and uterine epithelium prevails. This is particularly obvious in the rabbit illustrated here, where the mucoprotein layer formed already in the tubes is being reinforced by addition of two new layers, the neozona and the gloiolemma. It seems to apply also to other species (particularly those representing the central type of implantation) although they usually do not show distinct layers of the blastocyst coverings. For simplicity, it is assumed that only one neozona precursor protein (W) is being secreted by the trophoblast and cross-linked by an enzyme present at the interface, and that another protein (X) is being provided by the endometrium as a precursor for the gloiolemma. However, the number of proteins involved, their origin and the nature and origin of the cross-linking enzyme(s) are still unknown. (Three major glycoproteins are found for example in the zona pellucida of pig and rabbit oocytes).[38] It must be considered a possibility that lectin-like molecules diffusing against neozona precursor, or gloiolemma precursor, respectively, may bring about the initial precipitation at the inner and outer surface of the mucoprotein layer, the latter being rate-limiting for diffusion. Enzymatic cross-linking may then stabilize the newly formed layer subsequently. However, there is no proof of the presence of lectin-like molecules so far. In the *initiation phase* of implantation (**B**), the "barrier" (i.e., the blastocyst coverings) formed or reinforced during the preimplantation phase is being removed by the action of specific proteinases (e.g., blastolemmase). When proteinases are inhibited experimentally, contact formation between trophoblast and uterine epithelium is prevented. In such experiments it becomes obvious, at the same time, that the potential for depositing neozona and gloiolemma material is still present at this phase although in the physiologic situation it is overcome by the degradating action of blastolemmase. Trophoblast and uterine epithelium can come together after removal of the intervening extracellular material of the blastocyst coverings. A similar concept may apply for removal of some components of cell surface coats. Since blastolemmase is a highly specific enzyme, other (glyco-)proteins can be expected to remain undegraded, or only chemically modified due to limited proteolysis, at the cell surfaces. For simplicity, only one such protein (Y) is shown at the surface of the trophoblast and another one (Z) at the uterine epithelial surface. In the *adhesion phase* (**C**), contact is formed between the cell surfaces of the trophoblast and the apical end of the uterine epithelium. This may involve, in the beginning, relatively weak bonds (of various chemical types) between cell surface glycoproteins, but the contact gradually becomes more and more firm so that finally the blastocyst cannot be separated from the uterine epithelium without destroying cells. Intercellular junctions including desmosomes are formed between both partners at the end. It is hypothesized that cell surface-bound (glyco-)proteins like those mentioned above (which are blastolemmase-resistant) are being cross-linked in order to reinforce contact, perhaps using again an enzyme like the one involved in deposition of blastocyst coverings material (the latter, however, being blastolemmase-sensitive).

found to be regulated separately.[122] While this system may give useful information on adhesiveness and on some aspects of invasiveness in general, it suffers the drawbacks of two-dimensional systems (discussed in[86]), and it may not be a suitable model for the process of epithelial penetration in particular, because cells change many of their properties including the polarity typical for epithelial cells.[40]

A useful summary of the various modes of epithelial penetration as seen electron microscopically in different species was given by Schlafke and Enders.[115] The principal modes are (a) penetration by *displacement* of uterine epithelium from its basement membrane (perhaps a subtype of [c]) (rat, mouse); (b) penetration by *fusion* of trophoblast and uterine epithelial cells (rabbit); (c) penetration by *intrusion* of processes of trophoblast between uterine epithelial cells (ferret, others?). It is not known to which type the human belongs since the early phase of epithelial penetration has not been observed here. In a slightly later stage, evidence for fusion between trophoblast and endometrial cells has been found electron microscopically.[77] The paraffin sections of the Carnegie collection, still the best specimens of earliest human implantation stages, also suggest symplasma formation in part of the uterine epithelium adjacent to the invading trophoblast (although it is not known whether this is just a sign of degeneration), and small nuclei of quite similar appearance are seen in the periphery of the earliest syncytiotrophoblast. The human may represent a combination of the intrusion and the fusion type. The same may hold true for the rhesus monkey, although this species shows a number of peculiar features (such as central type of implantation and epithelial plaque reaction), which limit its use as a model for human implantation[41] (Denker, Enders and Schlafke, in preparation).

In any case, the fact that the trophoblast can interact at all with the apical end of the uterine epithelium (which, as in all epithelia, is primarily nonadhesive) is most astonishing. As seen in microcinematographs of in vitro cultures, other cells tend to withdraw from the advancing trophoblast.[52] In the receptive phase (hormonally controlled[108]) uterine epithelial cells in the rat and mouse tend to detach from their basement membrane not only upon contact with the mature trophoblast but even after mechanical irritation (for references see[25, 115]). In the rabbit, hemidesmosome-like structures were found to form between, astonishingly, the apical end of the uterine epithelium and remnants of blastocyst coverings when the dissolution of the latter was blocked with aprotinin.[25] Does all this indicate that, perhaps, the uterine epithelium loses or even reverses its functional (apical-basal) polarity, after proper hormonal conditioning, when the trophoblast contacts it? This might be one factor contributing to the introduction of changes in the properties of the apical cell surface of the uterine epithelium, to changes in transport, but also to changes in the lysosomal system, finally leading to cell death. It was indeed found that the rat uterine epithelium undergoes considerable changes in transport phenomena, in the preimplantation phase (also in pseudopregnancy), showing increased endocytotic activity.[42, 103] It was proposed that endocytosis and attachment may be related phenomena in molecular terms, attachment being "frustrated endocytosis or phagocytosis."[109, 124] Does this apply to the attachment between trophoblast and uterine epithelium?

The trophoblast does not seem to be affected by stimuli changing the functional polarity of uterine epithelial cells, in this respect once more resembling tumor cells that are not contact-inhibited. The acquisition of a stable polarity may be a major point in

trophoblast differentiation and maturation as discussed elsewhere.[29] Formation of a basement membrane underneath the trophoblast cells (in murine rodents attaining considerable thickness as Reichert's membrane, usually thought to control transport to the yolk sac), aided by the primary endoderm, may help in stabilizing this polarity. Basement membranes can imprint polarity on cells, even on certain tumor cells.[61]

Some information is available on the motility of mouse implantation stage trophoblast in vitro, including observations on actin and myosin.[52, 125] It has also been proposed to focus attention on its cytoskeletal organization in general.[122] However, it has rarely been asked specifically why the trophoblast invades the endometrium rather than the blastocyst cavity or the embryonic anlage? Further investigation of factors regulating cell polarity and directional growth may well shed new light on mechanisms of trophoblast invasion.

After the trophoblast has penetrated the uterine epithelium, invasion appears to be halted for a while before the *basement membrane* is overcome.[115] The mechanism of basement membrane penetration by the trophoblast is still unknown. Recent studies reveal that certain highly metastatic tumor cells produce collagenases specific for type IV collagen.[83] Such an enzyme has not yet been shown in the invading trophoblast. Alternatively, loss of functional polarity of uterine epithelial cells, as discussed above, can contribute to the process by reducing deposition of basement membrane material while the degradative activity of normal turnover is unchanged. The trophoblast is not always the first cell to penetrate the basal lamina: in the rat, ectoplasmic processes of stromal cells traverse it earlier.[44]

The spectrum of molecular events discussed here is still highly hypothetical and is simplified for heuristic purposes. The strongest evidence is available for the role of blastolemmase in the initiation phase of implantation. The other details proposed may help to design appropriate experiments to elucidate the mechanisms of implantation initiation further. We should be aware of the possibility that proteinase inhibitors used as an additive to medicated IUDs many affect implantation in addition to reducing menstrual blood loss. Different proteinase inhibitors may have quite different effects, depending on the particular proteinase attacked. While inhibitors of blastolemmase-type enzymes may interfere with implantation initiation not only in the rabbit but also in other species perhaps including the human, selective plasmin inhibitors may have an implantation-promoting effect if any. Blastolemmase-resistant proteins can be expected to be involved in mediating attachment, and they might be a promising tool in attempts to increase success rates in blastocyst transfer.

REFERENCES

1. Alving RE, Laki K: Enzymatic stabilization of protein aggregates. Fed Proc 26:828, 1967
2. Anderson WA, Desombre ER, Kang Y-H: Estrogen-progesterone antagonism with respect to specific marker protein synthesis and growth by the uterine endometrium. Biol Reprod 16:409–419, 1977
3. Andrade ATL, Shaw ST Jr, Guerra MO, Aaronson DE: Effects of epsilon-aminocaproic acid on fertility in the rabbit. J Reprod Fert 52:261–264, 1978
4. Beier HM: Hormonal stimulation of protease inhibitor activity in endometrial secretion during early pregnancy. Acta Endocrinol 63:141–149, 1970
5. Beier HM: Uteroglobin and related biochemical changes in the reproductive tract during early pregnancy in the rabbit. J Reprod Fert Suppl 25:53–69, 1976
6. Betteridge KJ, Eaglesome MD, Mitchell D, Flood PF, Bériault R: Development of horse embryos up to twenty-two days after ovulation: observations of fresh specimens. J Anat (in press)
7. Bohn H: Isolation and characterization of placental proteins with special reference to pregnancy-specific β_1 glycoprotein and other proteins specific to the placenta. In Klopper A, Chard T (eds): Placental Proteins. Berlin Heidelberg New York, Springer-Verlag, 1979, pp 71–88
8. Böving BG: Implantation mechanisms. In Hartman CG (ed): Conference on Physiological Mechanisms Concerned with Conception. Oxford, Pergamon Press, 1963, pp 321–396
9. Brandon JM: Some recent work on the role of histamine in ovum implantation. In Leroy F et al (eds): Blastocyst-Endometrium Relationships (Prog Reprod Biol 7) Basel, S Karger, 1980, pp 244–252
10. Carollo JR, Weitlauf HM: Regional changes in the binding of (^{3}H) concanavalin A to mouse blastocysts at implantation: an autoradiographic study. J Exp Zool 218:247–251, 1981
11. Casslén B: Proteinases and proteinase inhibitors in uterine fluid with special reference to IUD-users. Acta Obstet Gynecol Scand Suppl 98, 1981
12. Chandler DE, Heuser J: The vitelline layer of the sea urchin egg and its modification during fertilization. A freeze-fracture study using quick-freezing and deep-etching. J Cell Biol 84:618–632, 1980
13. Chávez DJ, Enders AC: Temporal changes in lectin binding of peri-implantation mouse blastocysts. Develop Biol 87:267–276, 1981
14. Chung SI: Comparative studies on tissue transglutaminase and factor XIII. Ann NY Acad Sci 202:240–255, 1972
15. Cornwell MM, Davies PJA, Juliano RL: Adhesion of CHO cells to a serum coated substratum is blocked by a transglutaminase inhibitor. J Cell Biol 87:62a, 1980
16. Dabich D, Andary TJ: Prevention of blastocyst implantation in mice with proteinase inhibitors. Fert Steril 25:954–957, 1974
17. Dabich D, Andary TJ: Tryptic- and chymotryptic-like proteinases in early and late preimplantation mouse blastocysts. Biochim Biophys Acta 444:147–153, 1976
18. Dabich D, Owens MS: Trypsin-like inhibitor activity in mouse uteri during early gestation and delayed implantation. Proc Soc Exp Biol Med 157:175–179, 1978
19. Denker H-W: Topochemie hochmolekularer Kohlenhydratsubstanzen in Frühentwicklung und Implantation des Kaninchens. I. Allgemeine Lokalisierung und Charakterisierung hochmolekularer Kohlenhydratsubstanzen in frühen Embryonalstadien. Zool Jahrb Abt allg Zool Physiol 75:141–245, 1970
20. Denker H-W: Topochemie hochmolekularer Kohlenhydratsubstanzen in Frühentwicklung

und Implantation des Kaninchens. II. Beiträge zu entwicklungsphysiologischen Fragestellungen. Zool Jahrb Abt allg Zool Physiol 75:246–308, 1970

21. Denker H-W: Enzym-Topochemie von Frühentwicklung und Implantation des Kaninchens. III. Proteasen. Histochemie 33:344–360, 1971

22. Denker H-W: Enzym-Topochemie von Frühentwicklung und Implantation des Kaninchens. II. Glykosidasen. Histochemie 33:268–285, 1971

23. Denker H-W: Blastocyst protease and implantation: Effect of ovariectomy and progesterone substitution in the rabbit. Acta endocr (Copenh) 70:591–602, 1972

24. Denker H-W: Trophoblastic factors involved in lysis of the blastocyst coverings and in implantation in the rabbit: Observations on inversely orientated blastocysts. J Embryol Exp Morph 32:739–748, 1974

25. Denker H-W: Implantation: The Role of Proteinases, and Blockage of Implantation by Proteinase Inhibitors. Berlin, Springer (= Adv Anat Embryol Cell Biol 53, Fasc 5) 1977

26. Denker H-W: Inhibitors of trophoblast proteinases. In Beller FK, Schumacher GFB (eds): The Biology of the Fluids of the Female Genital Tract. New York, Elsevier North-Holland, 1979, pp 151–161

27. Denker H-W: Role of proteinases in implantation. In Leroy F, Finn CA, Psychoyos A, Hubinont PO (eds): Blastocyst-Endometrium Relationships (Prog Reprod Biol 7) Basel, S Karger, 1980, pp 28–42

28. Denker H-W: Proteases of the blastocyst and of the uterus. In Beier HM, Karlson P (eds): Proteins and Steroids in Early Pregnancy. Berlin Heidelberg, Springer-Verlag, 1982, pp 183–208

29. Denker H-W: Cell lineage, determination and differentiation in earliest developmental stages in mammals. In W Hilscher (ed): Problems of the Keimbahn. Basel, S Karger Bibliotheca anat, 24, 1982

30. Denker H-W, Eng LA, Hamner CE: Studies on the early development and implantation in the cat. II. Implantation: proteinases. Anat Embryol 154:39–54, 1978

31. Denker H-W, Fritz H: Enzymic characterization of rabbit blastocyst proteinase with synthetic substrates of trypsin-like enzymes. Hoppe-Seyler's Z physiol Chem 360:107–113, 1979

32. Denker H-W, Gerdes H-J: The dynamic structure of rabbit blastocyst coverings. I. Transformation during regular preimplantation development. Anat Embryol 157:15–34, 1979

33. Denker H-W, Hafez ESE: Proteases and implantation in the rabbit: Role of trophoblast versus uterine secretion. Cytobiologie 11:101–109, 1975

34. Denker H-W, Petzoldt U: Proteinases involved in implantation initiation in the rabbit: Microdisc electrophoretic studies. Cytobiologie 15:363–371, 1977

35. Denker H-W, Tyndale-Biscoe CH: Implantation-associated proteinase activities in a marsupial (Macropus eugenii). Biol Reprod 24 Suppl 1:78A, 1981

36. Dey SK, Johnson DC: Reevaluation of histamine in implantation. In Kimball F (ed): The Endometrium. New York, Spectrum Publications, 1980, pp 269–283

37. Desombre ER, Lyttle CR: Induction and regulation of uterine peroxidase by steroid hormones. In Beato M (ed): Steroid Induced Uterine Proteins. Elsevier North-Holland, Biomedical Press, 1980, pp 283–296

38. Dunbar BS, Liu C, Sammons DW: Identification of the three major proteins of porcine and rabbit zonae pellucidae by high resolution two-dimensional gel electrophoresis: Comparison with serum, follicular fluid, and ovarian cell proteins. Biol Reprod 24:1111–1124, 1981

39. Enders AC: The fine structure of the blastocyst. In Blandau RJ (ed): The Biology of the Blastocyst. Chicago London, The University of Chicago Press, 1971, pp 71–94

40. Enders AC, Chávez DJ, Schlafke S: Comparison of implantation in utero and in vitro. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation. New York London, Plenum Press, 1981, pp 365–382

41. Enders AC, Hendricks AG: Morphologic basis of implantation in the rhesus monkey. In Leroy F, Finn CA, Psychoyos A, Hubinont PO (eds): Blastocyst-Endometrium Relationships (Prog Reprod Biol 7), 1980, pp 270–283

42. Enders AC, Nelson DM: Pinocytotic activity of the uterus of the rat. Am J Anat 138:277–300, 1973
43. Enders AC, Schlafke S: Surface coats of the mouse blastocyst and uterus during the preimplantation period. Anat Rec 180:31–46, 1974
44. Enders AC, Schlafke S: Comparative aspects of blastocyst-endometrial interactions at implantation. In Maternal Recognition of Pregnancy. Ciba Foundation Series 64 (new series), Excerpta Medica, Amsterdam, 1979, pp 3–32
45. Enders AC, Schlafke S: Differentiation of the blastocyst of the rhesus monkey. Am J Anat 162:1–21, 1981
46. Enders AC, Schlafke S, Welsh, AO: Trophoblastic and uterine luminal epithelial surfaces at the time of blastocyst adhesion in the rat. Am J Anat 159:59–72, 1980
47. Fazleabas AT: The noninvasive nature of placentation in the pig. Purification and properties of a progesterone-induced serine protease inhibitor from uterine secretions. Biology of Reproduction 24 Suppl 1, 1981, p 33A
48. Fischer B, Denker H-W, Beier HM: Zum Hüllenumbau von praeimplantatorischen Kaninchenembryonen in vitro. Zuchthygiene (in press)
49. Flint APF: A unifying hypothesis for the control of blastocyst growth based on observations of the pig. J Reprod Fert Suppl 29:215–227, 1981
50. Foley ME, McNicol GP: Production of fibrinolytic enzymes by macrophages on IUCDs. Br J Obstet Gynaec 85:551–556, 1978
51. Gerdes H-J: Die strukturelle Umgestaltung der extrazellulären Keimhüllen während der Frühentwicklung und Implantation beim Kaninchen. Dissertation, Med Fak RWTH Aachen (West Germany), 1980
52. Glass RH, Spindle AI, Pedersen RA: Mouse embryo attachment to substratum and interaction of trophoblast with cultured cells. J Exp Zool 208:327–336, 1979
53. Glasser SR, McCormack SA: Cellular and molecular aspects of decidualization and implantation. In Beier HM, Karlson P (eds): Proteins and Steroids in Early Pregnancy, Berlin Heidelberg New York, Springer-Verlag, 1982, pp 245–310
54. Grinnell F: Cell attachment to a substratum and cell surface proteases. Arch Biochem Biophys 169:474–482, 1975
55. Guillomot M, Fléchon JE, Wintenberger-Torrès S: Cellular contact between the trophoblast and the endometrium at implantation in the ewe. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation, New York London, Plenum Press, 1981, pp 446–449
56. Hackenberg R, Beier HM: Progesterone-stimulated protease inhibitor activity in uterine secretion of the rabbit. Acta endocr (Kbh) Suppl 246:5–6, 1982
57. Hewitt K, Beer AE, Grinnell F: Disappearance of anionic sites from the surface of the rat endometrial epithelium at the time of blastocyst implantation. Biol Reprod 21:691–707, 1979
58. Hicks JJ, Guzmán-Gonzáles AM: Inhibition of implantation by intraluminal administration of concanavalin A in mice. Contraception 20:129–136, 1979
59. Hoversland RC, Weitlauf HM: The effect of estrogen and progesterone on the level of amidase activity in fluid flushed from the uteri of ovariectomized mice. Biol Reprod 19:908–912, 1978
60. Hoversland RC, Weitlauf HM: Lysis of the zona pellucida and attachment of embryos to the uterine epithelium in ovariectomized mice treated with estradiol-17β and progesterone. J Reprod Fert 62:111–116, 1981
61. Ingber DE, Medri JA, Jamieson JD: The role of the basement membrane constituents, laminin and type IV collagen, in the organization of a tumor. J Cell Biol 87:127a, 1980
62. Ito A, Ihara H, Mori Y: Partial purification and characterization of a novel neutral proteinase from human uterine cervix. Biochem J 185:443–450, 1980
63. Jenkinson EJ: The in vitro blastocyst outgrowth system as a model for the analysis of periimplantation development. In Johnson MH (ed): Development in Mammals, vol. 2, Amsterdam New York Oxford, Elsevier North-Holland Publishing Company, 1977, pp 151–172
64. Jenkinson EJ, Searle RF: Cell surface changes on the mouse blastocyst at implantation. Exp Cell Res 106:386–390, 1977

65. Johnson MH: Studies using antibodies to the macromolecular secretions of the early pregnant uterus. In Centaro A, Carretti N (eds): Immunology in Obstetrics and Gynecology, Amsterdam, Excerpta Medica, 1974, pp 123–133

66. Jost V, Brökelmann J: Histochemischer Nachweis von Peroxydasen im Uterus des Menschen und verschiedener Tierarten. Anat Anz 147:503, 1980

67. Katz J, Troll W, Genunchi A, Levitz M: Prevention of normal mouse uterine maturation by antipain and elastatinal: Suppression of peroxidase activity. Endocrine Res Commun 5:325–334, 1978

68. Katz J, Troll W, Levy M, Filkins K, Russo J, Levitz M: Estrogen dependent trypsin-like activity in the rat uterus. Localization of activity in the 12,000 g pellet and nucleus. Arch Biochem Biophys 173:347–354, 1976

69. Kennedy TG, Armstrong DT: The role of prostaglandins in endometrial vascular changes at implantation. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation. New York, London, Plenum Press, 1981, pp 349–363

70. Kirchner C: Uterine protease activities and lysis of the blastocyst covering in the rabbit. J Embryol Exp Morph 28:177–183, 1972

71. Kirchner C, Hirschhäuser C, Kionke M: Protease activity in rabbit uterine secretion 24 hours before implantation. J Reprod Fert 27:259–260, 1971

72. Klebanoff SJ, Smith DC: Peroxidase-mediated antimicrobial activity of rat uterine fluid. Gynec Invest 1:21–30, 1970

73. Kramer RH, Vogel KG, Nicolson GL: Degradation of vascular endothelial extracellular matrix by metastatic tumor cells. J Cell Biol 87:98a, 1980

74. Kubo H, Spindle AI, Pederson RA: Possible involvement of protease in mouse blastocyst implantation in vitro. Biol Reprod 20 Suppl 1:50A, 1979

75. Kubo H, Spindle A, Pedersen RA: Inhibition of mouse blastocyst attachment and outgrowth by protease inhibitors. J Exp Zool 216:445–451, 1981

76. Kuettner KI, Pauli BU: Resistance of cartilage to normal and neoplastic invasion. In Horton JE et al (eds): Proceedings, Mechanisms of Localized Bone Loss. Special Suppl to Calcified Tissue Abstracts, 1978, pp 251–278

77. Larsen JF: Human implantation and clinical aspects. In Leroy F, Finn CA, Psychoyos A, Hubinont PO (eds): Blastocyst-Endometrium Relationships (Prog Reprod Biol, vol 7), Basel München Paris London New York Sydney, S Karger, 1980, pp 284–295

78. Larsson B: Experimental and clinical studies on the copper-7-intrauterine device. Thesis, Göteborg, 1975

79. Larsson B, Liedholm P, Åstedt B: Increased fibrinolytic activity in the endometrium of patients using copper-IUD (Gravigard). Int J Fert 20:77–80, 1975

80. Laki K: Faktor XIII und Metastasierung. Thrombos diathes haemorrh Suppl 62:61–71, 1976

81. Ledger WJ, Virkar KD, Irvin LA: Effect of a plastic intrauterine device upon implantation in the rabbit. Obstet Gynecol 28:521–525, 1966

82. Liedholm P, Åstedt B: Fibrinolytic activity of the rat ovum, appearance during tubal passage and disappearance at implantation. Int J Fertil 20:24–26, 1975

83. Liotta LA, Tryggvason K, Garbisa S, Gehron Robey P, Murray JC: Interaction of metastatic tumor cells with basement membrane collagen. In Grundmann E (ed): Metastatic Tumor Growth. Stuttgart New York, Gustav Fischer Verlag, 1980, pp 21–30

84. Magnuson T, Epstein CJ: Characterization of concanavalin A precipitated proteins from early mouse embryos: A 2-dimensional gel electrophoresis study. Develop Biol 81:193–199, 1981

85. Mancarella DA, Basha SMM, Mullins DE, Mansfield E, Bazer FW, Roberts RM: Properties of a membrane-associated L-leucine β-naphthylamidase (leucine aminopeptidase) from the porcine uterus. Biol Reprod 24:879–887, 1981

86. Mareel MM: Recent apsects of tumor invasiveness. Int Rev Exp Pathol 22:65–129, 1980

87. Martin L: What roles are fulfilled by uterine epithelial components in implantation? In Leroy F, Finn CA, Psychoyos A, Hubinont PO (eds): Blastocyst-Endometrium Relationships. Basel München Paris London New York Sydney, S Karger, 1980, pp 54–69

88. McLaren A: The fate of the zona pellucida in mice. J Embryol Exp Morph 23:1–19, 1970

89. McLaren A: Endocrinology of implantation. Abstract, 1st South-East Asia and Oceana Med Sci Congr Int Plann Parent Fedn Sydney, 1972, p 16

90. Meinshausen E: Beeinflussung der Embryo-Implantation durch fortlaufende intrauterine Verabreichung eines Proteinaseinhibitors. Experimentelle Untersuchungen am Kaninchen. Dissertation, Med Fak RWTH Aachen (West Germany), 1982

91. Mintz B: Implantation-initiating factor from mouse uterus. In Moghissi KS, Hafez ESE (eds): Biology of Mammalian Fertilization and Implantation, Springfield Illinois, CC Thomas, 1972, pp 343–356

92. Mosher DF, Vaheri A: Thrombin stimulates the production and release of a major surface-associated glycoprotein (fibronectin) in cultures of human fibroblasts. Exp Cell Res 112:323–334, 1978

93. Moulton BC, Elangovan S: Lysosomal mechanisms in blastocyst implantation and early decidualization. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation. New York London, Plenum Press, 1981, pp 335–344

94. Mukherjee AB, Laki K, Agrawal AK: Possible mechanism of success of an allotransplantation in nature: Mammalian pregnancy. Medical Hypotheses 6:1043–1055, 1980

95. Mullins DE, Bazer FW, Roberts RM: The pig uterus secretes a progesterone-induced inhibitor of plasminogen activator. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation. New York London, Plenum Press, 1981, pp 420–422

96. Nakai M, Atsumi M, Magima T, Arai M: Presence of a trypsin-like protease in the human placenta: Isolation and characterization. Biol Reprod 18 Suppl 1, 37A, 1978

97. Nilsson O: Local secretory response by the mouse uterine epithelium to the presence of a blastocyst or a blastocyst-like bead. Anat Embryol 150:313–318, 1977

98. Nilsson O, Lindqvist I, Ronquist G: Decreased surface charge of mouse blastocysts at implantation. Exp Cell Res 83:421–423, 1973

99. Nilsson BO, Naeslund G, Curman B: Polar differences of delayed and implanting mouse blastocysts in binding of Alcian Blue and concanavalin A. J Exp Zool 214:177–180, 1980

100. Notides AC, Hamilton DE, Rudolph JH: The action of a human uterine protease on the estrogen receptor. Endocrinology 93:210–216, 1973

101. Ortolani G, Patricolo E, Mansueto C: Trypsin-induced cell surface changes in ascidian embryonic cells. Regulation and differentiation of a tissue-specifc protein. Exp Cell Res 122:137–147, 1979

102. Owers NO, Blandau RJ: Proteolytic activity of the rat and guinea pig blastocyst in vitro. In Blandau RJ (ed): The Biology of the Blastocyst. Chicago, University of Chicago Press, 1971, pp 207–223

103. Parr MB: Endocytosis in the uterine epithelium during early pregnancy. In Leroy F, Finn CA, Psychoyos A, Hubinont PO (eds): Blastocyst-Endometrium Relationships (Prog Reprod Biol, vol 7) Basel München Paris London New York Sydney, S Karger, 1980, pp 81–91

104. Petzoldt U: Untersuchung über das anorganische Milieu in Uterus und Blastozyste des Kaninchens. Zool Jahrb Abt Allg Zool u Physiol 75:547–593, 1971

105. Pietras RJ, Szego CM: Estrogen-induced membrane alterations and growth associated with proteinase activity in endometrial cells. J Cell Biol 81:649–663, 1979

106. Pinsker MC, Sacco AG, Mintz B: Implantation-associated proteinase in mouse uterine fluid. Develop Biol 38:285–290, 1974

107. Porter CW: Neuraminidase treatments and implantation in mice. Int J Fertil 20:194–196, 1975

108. Psychoyos A, Casimiri V: Factors involved in uterine receptivity and refractoriness. In Leroy F, Finn CA, Psychoyos A, Hubinont PO (eds): Blastocyst-Endometrium Relationships (Prog Reprod Biol, vol 7) Basel München Paris London New York Sydney, S Karger, 1980, pp 143–157

109. Rajaraman R, MacSween JM, Fox RA: Models of normal and transformed cell adhesion, and capping and locomotion in vitro. J Ther Biol 74:177–201, 1978

110. Renfree MB: The composition of fetal fluids of the marsupial *Macropus eugenii*. Develop Biol 33:62–79, 1973

111. Roberts RM, Bazer FW: The properties, function and hormonal control of synthesis of uteroferrin, the purple protein of the pig uterus. In Beato M (ed): Steroid Induced Uterine Proteins. Amsterdam New York Oxford, Elsevier North-Holland Biomedical Press, 1980, pp 133-149

112. Roseman S: Complex carbohydrates and intercellular adhesion. In Moscona AA (ed): The Cell Surface in Development. New York London Sydney Toronto, John Wiley & Sons, 1974, pp 255-271

113. Rosenfeld MG, Joshi MS: The effect of estrogen and progesterone on the appearance of a uterine specific peptidase in mammals. Anat Rec 184:593, 1976

114. Rosenfeld MG, Joshi MS: Role of a uterine endopeptidase in the implantation process of the rat. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation. New York London, Plenum Press, 1981, pp 423-427

115. Schlafke S, Enders AC: Cellular basis of interaction between trophoblast and uterus at implantation. Biol Reprod 12:41-65, 1975

116. Schmell ED, Gulyas BJ: Ovoperoxidase activity in ionophore treated mouse eggs. II. Evidence for the enzyme's role in hardening the zona pellucida. Gamete Research 3:279-290, 1980

117. Schmidt-Matthiesen H: Die fibrinolytische Aktivität von Endometrium und Myometrium, Decidua und Plazenta, Kollum- und Korpuskarzinomen. Physiologie, Pathologie und klinisch-therapeutische Konsequenzen. Fortschr Geburtsh Gyn 31, Basel New York, S Karger, 1967

118. Schumacher GFB, Holt JA, Reale F: Approaches to the analysis of human endometrial secretions. In Beller FK, Schumacher GFB (eds): The Biology of the Fluids of the Female Genital Tract. New York, Elsevier North Holland Inc 1979, pp 115-130

119. Schwick HG: Chemisch-entwicklungsphysiologische Beziehungen von Uterus zu Blasto-cyste des Kaninchens Oryctolagus cuniculus. Roux' Archiv für Entwicklungsmechanik 156:283-343, 1965

120. Shapiro BM, Schackmann RW, Gabel C: Molecular approaches to the study of fertilization. Ann Rev Biochem 50:815-843, 1981

121. Sherman MI: Studies on the temporal correlation between secretion of plasminogen activator and stages of early mouse embryogenesis. Oncodevelopmental Biol Med 1:7-17, 1980

122. Sherman MI, Matthaei KI: Factors involved in implantation-related events in vitro. In Leroy F, et al (eds): Blastocyst-Endometrium Relationships. (Prog Reprod Biol, vol 7) Basel München Paris London New York Sydney, S Karger 1980, pp 43-53

123. Sherman MI, Sellens MH, Atienza-Samols SB, Pai AC, Schindler J: Relationship between programs for implantation and trophoblast differentiation. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation. New York, Plenum, 1981, pp 75-89

124. Smolen JE, Weissmann G: The granulocyte: metabolic properties and mechanisms of lysosomal enzyme release. In Havemann K, Janoff A (eds): Neutral proteases of Human Polymorphonuclear Leukocytes. Baltimore Munich, Urban Schwarzenberg Verlag, 1978, pp 56-76

125. Sobel JS, Glass RH, Cooke R, Spindle AI, Pedersen RA: Cell motility and the distribution of actin and myosin during mouse trophoblast development in vitro. In Glasser SR, Bullock DW (eds): Cellular and Molecular Aspects of Implantation. New York London, Plenum Press, 1981, pp 450-453

126. Graf von Spee F: Beitrag zur Entwickelungsgeschichte der frühen Stadien des Meerschweinchens bis zur Vollendung der Keimblase. Arch Anat Entwickl-Gesch 1883:44-60

127. Graf von Spee F: Die Implantation des Meerschweincheneies in die Uteruswand. Z Morph Anthropol 3:130-182, 1901

128. Strickland S, Reich E, Sherman MJ: Plasminogen activator in early embryogenesis: Enzyme production by trophoblast and parietal endoderm. Cell 9:231-240, 1976

129. Surani MAH, Fishel SB: Blastocyst-uterine interactions at implantation. In Leroy F et al (eds): Blastocyst-Endometrium Relationships. (Prog Reprod Biol, vol 7) Basel München Paris London New York Sydney, S Karger, 1980, pp 14-27

130. Tauber PF: Immunproteine und Enzyme im Endometrium und in uterinen Sekreten. Ein Beitrag zur Physiologie und Pathophysiologie der uterinen Wundheilung post partum und der intrauterinen Kontrazeption. Stuttgart New York, FK Schattauer Verlag, 1981

131. Tauber PF, Wolf AS, Herting W, Zaneveld LJD: Hemorrhage induced by intrauterine devices: Control by local proteinase inhibition. Fert Ster 28:1375–1377, 1977

132. Thomas WA, Thomson J, Magnani JL, Steinberg MS: Two distinct adhesion mechanisms in embryonic neural retina cells. III. Functional specificity. Develop Biol 81:379–385, 1981

133. Tzartos SJ, Surani MAH: Affinity of uterine luminal proteins for rat blastocysts. J Reprod Fert 56:579–586, 1979

134. Unger T, Struck H: Proteolytische Enzymaktivitäten der reifen menschlichen Plazenta. II. Neutrale Peptidasen und PZ-Peptidase. Arch Gynäk 225:227–235, 1978

135. van Hoorn G, Denker H-W: Effect of the blastocyst on a uterine amino acid arylamidase in the rabbit. J Reprod Fert 45:359–362, 1975

136. Waxler B, Kuettner KE, Memoli VA, Pauli BU: Anti-invasive factor(s) derived from bovine urinary bladder. J Cell Biol 87:116a, 1980

137. Weiss G, Beller FK: Tissue activator of the fibrinolytic enzyme in the female reproductive system. Obstet Gynecol 34:809–819, 1969

138. Weitlauf HM: Factors in mouse uterine fluid that inhibit the incorporation of (^{3}H)uridine by blastocysts in vitro. J Reprod Fert 52:321–325, 1978

139. Werb Z: Pathways for the modulation of macrophage collagenase activity. In Horton JE et al (eds): Mechanisms of Localized Bone Loss, Special Suppl to Calcified Tissue Abstracts, 1978, pp 213–228

140. Werb Z, Banda MJ, Jones PA: Degradation of connective tissue matrices by macrophages. I. Proteolysis of elastin, glycoproteins, and collagen by proteinases isolated from macrophages. J Exp Med 152:1340–1357, 1980

141. Werb Z, Glass R, Aggeler J: Interaction of mouse trophoblast with extracellular matrices—A model for embryo implantation. J Cell Biol 87:138a, 1980

142. WHO: Special Programme of Research Development and Research Training in Human Reproduction. 7th Annual Report, 1978

143. Wolf DP, Nishihara T, West DM, Wyrick RE, Hedrick JL: Isolation, physicochemical properties, and the macromolecular composition of the vitelline and fertilization envelopes from *Xenopus laevis* eggs. Biochem 15:3671–3678, 1976

144. Wood C, Psychoyos A: Activité de certaines enzymes hydrolytiques dans l'endomètre et le myomètre au cours de la pseudogestation et de divers états de réceptivité utérine chez la ratte. C R Acad Sci (Paris) Sér D 265:141–144, 1967

145. Wood JC, Williams EA, Barley VL, Cowdell RH: The activity of hydrolytic enzymes in the human endometrium during the menstrual cycle. J Obstet Gynaec Brit Cwlth 76:724–728, 1969

146. Wu JT: Concanavalin A binding capacity of preimplantation mouse embryos. J Reprod Fert 58:455–461, 1980

147. Wu JT, Chang MC: Increase in concanavalin A binding sites in mouse blastocysts during implantation. J Exp Zool 205:447–453, 1978

OBSTETRICS AND GYNECOLOGY ANNUAL

VOLUME 12

1983

SERIES EDITOR

RALPH M. WYNN, M.D.

Vice President for Medical Affairs, Ravenswood Hospital Medical Center, Chicago, Illinois