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Embryonic Signals and the Initiation of Blastocyst Implantation

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Abstract

The earliest sign of blastocyst implantation is an increase in endometrial vascular permeability which is localized in areas adjacent to blastocysts. The localized nature of this response suggests that it occurs in response to a signal from the blastocyst. It has been suggested that this signal may be physical in nature, or may be due to blastocyst-produced histamine, oestrogen or prostaglandins. The evidence for each of these is reviewed. At present, it is not possible to exclude any of these signals, which are not mutually exclusive, with certainty. The bulk of the evidence suggests that prostaglandins have an obligatory role in the initiation of implantation, but they may not necessarily be of blastocyst origin.

Introduction

An embryo is said to be implanted when it becomes fixed in position within the uterus, and establishes physical contact with the maternal organism (McLaren 1972). The manner in which the embryo implants varies among species (Psychoyos 1973; Finn and Porter 1975). In all cases, however, the embryo–endometrial interactions can only be initiated when the embryo and endometrium have reached a precise stage of maturity; the embryo has to be at the blastocyst stage, and hormone-dependent changes leading to the development of a 'receptive' endometrium must have occurred. Failure of synchronization in the development of the embryo and endometrium results in the failure or postponement of implantation.

In all species which have been investigated—with the possible exception of the pig (Heap *et al.* 1979)—the earliest macroscopically identifiable sign of blastocyst implantation is an increase in endometrial vascular permeability which is localized to the areas adjacent to the blastocysts (Psychoyos 1973). This increase in permeability is usually taken as defining the beginning of implantation, and is thought to be an essential prerequisite for implantation (Psychoyos 1973). In species in which endometrial stromal cells differentiate into decidual cells (decidualization) in response to artificial stimuli, an increase in endometrial vascular permeability precedes the differentiation of decidual cells (Psychoyos 1973).

The localized nature of the endometrial vascular permeability increase at implantation suggests that it occurs in response to a signal from the blastocyst. What is this signal? As an answer to this question, signals which have been proposed are (i) physical, (ii) histamine, (iii) blastocyst-produced oestrogen, and (iv) prostaglandins. These proposed signals, upon which this review will concentrate, are by no means mutually exclusive.

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Evidence for a Physical Signal

It has been clear since the classical experiments of Loeb (1910) that physical stimuli can mimic the presence of a blastocyst and produce decidualization. Since then, a large number of different stimuli have been found effective in producing an increase in endometrial vascular permeability and subsequent decidualization provided that the stimulus is given to an appropriately primed uterus (Psychoyos 1973; Finn and Porter 1975). However, the fact that physical stimuli can increase endometrial vascular permeability and bring about decidualization does not mean that the primary signal from the blastocyst is physical in nature; presumably this conclusion can only be arrived at after all chemical signals of blastocyst origin have been eliminated.

Ultrastructural studies of the early stages of implantation have suggested the possibility of physical interactions between trophoblastic cells and endometrial luminal epithelial cells. The endometrial permeability changes are preceded by interdigitation of microvilli on trophoblastic and epithelial cells, followed by broad areas of apposition of cell membranes (Parkening 1976; Enders and Schlafke 1979; Segalen and Chambon 1983). This intimate contact between trophoblastic and epithelial cells may be sufficient to signal the presence of the blastocyst. If this is the case, it would be necessary for the epithelial cells to convey the signal to the endometrial stroma, since it is the stroma and not the luminal epithelium which is vascularized. Presumably this would be achieved by the conversion of the physical signal to a chemical one by the epithelial cells. The importance of the luminal epithelium for decidualization has been indicated by the work of Ferrando and Nalbandov (1968) and Lejeune *et al.* (1981) which demonstrated that if the luminal epithelium is destroyed or removed, decidualization cannot be obtained in response to stimuli which would otherwise be deciduogenic. These results are consistent with the hypothesis that the luminal epithelium responds to natural and artificial deciduogenic stimuli with the production of a compound which then triggers decidualization. At present, it is not known if a change in endometrial vascular permeability can be obtained in the absence of the luminal epithelium.

The blastocyst may play a more active role in physically perturbing the luminal epithelium than is indicated by morphological studies. When cultured *in vitro*, rat and mouse blastocysts undergo repetitive contractions and dilatations (Cole 1967; Bitton-Casimiri *et al.* 1970; Hurst and MacFarlane 1981). If these contractions and dilatations also occur *in vivo*, it is possible that they could augment the physical signal attributable to the close contact between the blastocyst and uterine epithelium.

Histamine as the Signal

The possibility that histamine is the physiological inducer of decidualization was first proposed by Shelesnyak (1957) and has received considerable attention. The early work has been critically reviewed by De Feo (1967) and Humphrey and Martin (1968). Recently, as the result of the development of histamine H_1- and H_2-receptor antagonists, as well as inhibitors of histamine synthesis, interest in histamine as a mediator of implantation and decidualization has resurfaced.

Brandon and Wallis (1977) reported that when rats were treated with both histamine H_1- and H_2-receptor antagonists, the initiation of implantation, as indicated by the number and intensity of uterine dye sites (indicative of increased endometrial vascular permeability), was adversely affected. However, when a more potent H_2-receptor antagonist, metiamide, was substituted for burimamide, the initiation of implantation was not affected (Brandon and Raval 1979), a result which has been interpreted by Brandon (1980) as suggesting that the effect of burimamide seen in the original study (Brandon and Wallis 1977) was not mediated by H_2 receptors.

Rabbit and mouse blastocysts are capable of histamine synthesis (Dey *et al.* 1979*a*; Dey and Johnson 1980*a*) and in rabbits the injection into the uterine lumen of an inhibitor of histidine decarboxylase, the enzyme which converts histidine to histamine, interrupts implantation (Dey *et al.* 1979*a*; Dey 1981). Implantation in rabbits is also adversely affected by the intrauterine instillation of disodium cromoglycate, an inhibitor of histamine release from mast cells (Dey *et al.* 1978). In rats with delayed implantation, concomitant administration of histamine and oestradiol reduces the amount of oestradiol required to bring about implantation (Johnson and Dey 1980).

Dey *et al.* (1979*b*) have reported that rabbit blastocysts and endometrial cells have H_2 and H_1 receptors respectively. These workers suggest that the endometrial H_1 receptors mediate pro-inflammatory events such as increased vascular permeability and vasodilation whereas activation of the H_2 receptor may be involved in immunosuppressive effects.

Dey and Johnson (1980*b*) have incorporated these recent findings into a hypothetical model which attempts to explain the relationship between histamine and implantation. Central to this scheme is the proposal that histamine of blastocyst origin acts within the endometrium, possibly by stimulating prostaglandin production, to initiate implantation. This model is not able to explain readily the vascular permeability changes which precede artificially induced decidualization since in this situation an embryonic source of histamine is usually not present. In addition, contrary to what would be expected from the model, Hoffman *et al.* (1977) failed to induce decidualization when implants which released histamine were placed within the rabbit uterus.

Blastocyst-produced Oestrogen as a Signal

The proposal that blastocyst-produced oestrogen is involved in the initiation of implantation is an attractive hypothesis from the viewpoint that it has the potential of resolving one of the enigmas concerning the endocrine requirements for implantation. Why do some species require both oestrogen and progesterone of maternal origin for implantation while others require only progesterone? Perhaps, as proposed by Dickmann *et al.* (1976), all species require both steroid hormones, with the only difference between species being the relative importance of maternal and embryonic sources of oestrogen.

Experimental evidence for the involvement of blastocyst-produced oestrogen in the initiation of implantation may be summarized as follows (for references, see Dickmann *et al.* 1976, unless otherwise indicated):

1. In rabbit, rat, hamster and mouse blastocysts, 3β-hydroxy-Δ^5-steroid dehydrogenase, a key enzyme in the synthesis of steroid hormones, and β-hydroxysteroid dehydrogenase, the enzyme which interconverts oestrone and oestradiol, have been demonstrated by histochemical techniques. These observations have been interpreted as suggesting that these blastocysts can produce oestrogens.
2. Definitive evidence for aromatase (oestrogen synthetase) activity and oestrogen synthesis is available for a number of species (see Heap *et al.* 1981).
3. Blastocysts contain steroids.
4. Embryos at the 2- to 4-cell stage, incubated in a solution of oestradiol, induced a localized increase in endometrial vascular permeability when transferred to pseudopregnant rats whereas control transfers of embryos incubated in an oestradiol-free medium failed to do so (Dickmann *et al.* 1977).
5. Culture of mouse blastocysts in an anti-oestrogen prior to transfer to pseudopregnant hosts reduces the rate of implantation (Sengupta *et al.* 1981*a*), as does the instillation of an anti-oestrogen into the uterine lumen of hamsters (Sengupta *et al.* 1981*b*).
6. There is a decreased uterine uptake of labelled oestradiol at implantation sites, compared with interimplantation sites, during normal pregnancy (Sartor 1977) or after oestrogen-induced initiation of implantation in 'delayed-implanting' rats (Ward *et al.* 1978). In addition, Logeat *et al.* (1980) have reported that nuclear oestradiol

receptors are present in twofold higher concentrations in implantation sites than in interimplantation sites in rats. These data have been interpreted as supporting the hypothesis that steroids originating from the blastocyst act locally to affect the implantation site.

Not all evidence favours the proposal that blastocyst-produced oestrogen is involved in the initiation of implantation. Evidence against the hypothesis may be summarized as follows:

1. Histochemically demonstrable 3β-hydroxy-Δ^5-steroid dehydrogenase activity has been found in unfertilized hamster eggs which do not implant (Niimura and Ishida 1976). In addition, Bleau (1981) has suggested that the histochemical technique does not measure 3β-hydroxy-Δ^5-steroid dehydrogenase activity in rabbit blastocysts.
2. Bullock (1977) and Findlay (1983) have emphasized that the presence of an enzyme does not necessarily indicate that it has functional activity.
3. Evidence has been presented which strongly suggests that the steroid content of rabbit blastocysts is of maternal origin (Borland *et al.* 1977; Fujimoto and Sundaram 1978; Singh and Booth 1979).
4. Prior to implantation, synthesis or metabolism of steriods by mouse (Sherman and Atienza 1977) or rat (Marcal *et al.* 1975) embryos could not be detected.
5. In hamsters, neither an aromatase inhibitor (Brodie *et al.* 1978) nor inhibitors of steroidogenesis (Evans and Kennedy 1980) prevented the initiation of implantation, suggesting that blastocyst oestrogen production is not essential.
6. Martel and Psychoyos (1981) have reported a substantial decrease in the concentration of endometrial cytoplasmic oestradiol receptor, without a change in nuclear concentration, in the implantation sites of rats. These observations may explain the decreased uptake of labelled oestradiol reported by Sartor (1977) and Ward *et al.* (1978). In addition, Martel and Psychoyos (1981) have suggested that the higher nuclear concentrations of oestradiol receptors reported by Logeat *et al.* (1980) for implantation sites was due to their use of Trypan blue, which binds steroids, to identify the sites.
7. The blastocyst-produced oestrogen hypothesis cannot readily explain the increased endometrial vascular permeability and subsequent decidualization induced by artificial stimuli.

From the evidence at present available, it seems that an obligatory role of blastocyst oestrogen production in the initiation of implantation has not been firmly established. In those species where there is definitive biochemical evidence for oestrogen production by blastocysts, it has been suggested that this oestrogen is involved in the maternal recognition of pregnancy (Flint *et al.* 1979; Heap *et al.* 1981; Findlay 1983).

Prostaglandins as a Signal

Evidence

Considerable experimental data have recently accumulated which suggests that prostaglandins have an obligatory role in endometrial vascular permeability changes and subsequent decidualization in several species. In pregnant animals, indomethacin, an inhibitor of prostaglandin biosynthesis, delays or inhibits the localized increase in endometrial vascular permeability (rats: Kennedy 1977; Phillips and Poyser 1981; mice: Lundkvist and Nilsson 1980; hamsters: Evans and Kennedy 1978; rabbits: Hoffman *et al.* 1978) and implantation (rats: Gavin *et al.* 1974; mice: Lau *et al.* 1973; Saksena *et al.* 1976; Holmes and Gordashko 1980; rabbits: El-Banna 1980). The intrauterine administration of prostaglandin antagonists at the expected time of implantation reduces the number of implantation sites (Biggers *et al.* 1981). The concentrations of prostaglandins are elevated in the areas of increased endometrial vascular permeability (rats: Kennedy 1977; Kennedy and Zamecnik 1978; hamsters: Evans and Kennedy 1978; rabbits: Sharma 1979), and

exogenous prostaglandins can reverse, at least partially, the effects of indomethacin on implantation (rats: Oettel *et al.* 1979; mice: Saksena *et al.* 1976; Holmes and Gordashko 1980).

In non-pregnant animals with uteri sensitized for the decidual cell reaction, artificially induced decidualization is reduced by indomethacin administration (rats: Castracane *et al.* 1974; Tobert 1976; Sananes *et al.* 1976, 1981; Kennedy and Lukash 1982; mice: Rankin *et al.* 1979; Buxton and Murdoch 1982), as is the change in endometrial vascular permeability (Kennedy 1979). Uterine concentrations of prostaglandins are elevated by artificial deciduogenic stimuli before there are detectable changes in permeability (rats: Kennedy 1979, 1980*a*, 1980*b*; Kennedy *et al.* 1980; mice: Rankin *et al.* 1979; Milligan and Lytton 1982) and prostaglandins given into the uterine lumen of animals in which endogenous prostaglandin production has been inhibited can increase endometrial vascular permeability (Kennedy 1979, 1980*a*, 1980*b*; Kennedy and Lukash 1982) and bring about decidualization (Kennedy and Lukash 1982; Miller and O'Morchoe 1982).

Source of Prostaglandins

The source of the prostaglandins which are involved in the initiation of blastocyst implantation is uncertain; the two most likely sources are the blastocysts themselves and the endometrium.

The blastocysts as the source of prostaglandins could readily explain the localized nature of the permeability response. Prostaglandin synthesis by blastocysts of a number of species has been reported (rabbit: Dey *et al.* 1980; Harper *et al.* 1983; pig: Watson and Patek 1979; cow: Shemesh *et al.* 1979; Lewis *et al.* 1982; sheep: Marcus 1981; Hyland *et al.* 1982; Lacroix and Kann 1982). In all these species, the blastocysts undergo marked expansion prior to implantation. In contrast, attempts to demonstrate prostaglandin synthesis by rat (Kennedy and Armstrong 1981) or mouse (Racowsky and Biggers 1983) blastocysts, which remain small prior to implantation, have been unsuccessful, although the inhibition of hatching of mouse blastocysts *in vitro* by inhibitors of prostaglandin synthesis (Biggers *et al.* 1978; Baskar *et al.* 1981; Hurst and MacFarlane 1981) provides indirect evidence for its occurrence. However, demonstration of blastocyst prostaglandin production by itself is not sufficient; it is necessary to demonstrate that these prostaglandins act on the endometrium. As suggested by Biggers *et al.* (1978), blastocyst-produced prostaglandins may have functions within the blastocyst.

The endometrial cells as a source of prostaglandins regulating endometrial vascular permeability is an attractive hypothesis since it is capable of explaining the increase in permeability brought about by both blastocysts and artificial stimuli. As suggested by Kennedy (1980*c*), it is possible that blastocysts, as a result of their interaction with the endometrial luminal epithelium (see above), and artificial stimuli have the common property of 'traumatizing' the endometrium, thereby stimulating prostaglandin production. Artificial deciduogenic stimuli cause tissue damage (Finn 1977; Lundkvist *et al.* 1977) and in other tissues, injury is known to stimulate prostaglandin synthesis (Ramwell and Shaw 1970; Piper and Vane 1971).

If during the initiation of implantation, the endometrium produces prostaglandins in response to a physical signal from the blastocysts, which endometrial cells produce the prostaglandins? If the luminal epithelial cells are not a source of the prostaglandins, it would be necessary for the physical signal from the blastocyst to be transferred across the epithelium to act on the underlying stromal cells; this would presumably require the transformation of a physical signal to a chemical signal which stimulates prostaglandin production. Possibly of relevance to these considerations is the observation by Boshier (1976) that there is a localized depletion of neutral lipids from the epithelium surrounding rat blastocysts. These neutral lipids are mainly triacylglycerols (Boshier *et al.* 1981) and, if they contain arachidonic acid, their depletion may represent mobilization of stored

precursor for prostaglandin biosynthesis. Thus the prostaglandins may be produced within the luminal epithelium in response to either a physical or chemical signal from the blastocyst, and then diffuse into the endometrial stroma to bring about their effects. Alternatively, they may be produced within the stroma in response to a chemical signal which arises either from the epithelium (as a result of its interaction with the blastocyst) or from the blastocyst.

Which Prostaglandins?

Attempts to identify the prostaglandins involved in mediating the endometrial vascular permeability increase at implantation and following the application of artificial deciduogenic stimulus have relied upon uterine prostaglandin measurements and uterine responses to exogenous prostaglandins.

The concentrations of prostaglandins E, F and I_2 (measured as 6-oxo-prostaglandin $F_{1\alpha}$) are elevated at implantation sites (rat: Kennedy 1977; Kennedy and Zamecnik 1978; rabbit: Sharma 1979; Pakrasi and Dey 1982) and in the uterus following the application of an artificial deciduogenic stimulus (rat: Kennedy 1979, 1980*a*, 1980*b*; Kennedy *et al.* 1980; mouse: Jonsson *et al.* 1979; Rankin *et al.* 1979). Not all data are in agreement; in the hamster, prostaglandins E, but not F, concentrations are elevated at implantation sites (Evans and Kennedy 1978) while in the mouse, uterine concentrations of prostaglandins F, but not E or I_2, are elevated in response to a deciduogenic stimulus (Milligan and Lytton 1983). Thus measurements of prostaglandins have not identified a single prostaglandin as the mediator of the endometrial vascular changes.

The effects of exogenous prostaglandins have been no more enlightening than their measurements. Indirect evidence that prostaglandin $F_{2\alpha}$ may be involved has come from Saksena *et al.* (1976) and Oettel *et al.* (1979) who reported the induction of implantation with this prostaglandin in mice and rats, respectively. However, when applied locally into the uterine lumen, prostaglandin $F_{2\alpha}$ was found to be less effective than prostaglandin E_2 at inducing implantation in mice (Holmes and Gordashko 1980). Intrauterine administration of prostaglandin $F_{2\alpha}$ results in decidualization in rats (Sananes *et al.* 1976) and rabbits (Hoffman *et al.* 1977), although in the latter study prostaglandin E_2 was more effective than prostaglandin $F_{2\alpha}$. However, in these studies, since no inhibitors of endogenous prostaglandin production were used, it is not known if the reported responses were due to the exogenous prostaglandin, endogenously produced prostaglandin, or an interaction between exogenous and endogenous prostaglandins. In this regard, while Sananes *et al.* (1981) found prostaglandin $F_{2\alpha}$ to be effective in inducing decidualization when given to animals not treated with indomethacin, it was ineffective when given with indomethacin. To circumvent these problems of interpretation of responses, Kennedy and Lukash (1982) infused prostaglandins into the uterine lumen of rats which were treated with indomethacin to inhibit endogenous prostaglandin production during the initiation of the response. Prostaglandins E_2 and $F_{2\alpha}$, alone or combined, were effective in bringing about an increase in endometrial vascular permeability and decidualization and, at least in terms of the latter response, were equally potent. By contrast, when injected into the uterine lumen of indomethacin-treated rats, not only was prostaglandin $F_{2\alpha}$ by itself ineffective, but when administered with prostaglandin E_2, it inhibited the permeability response to prostaglandin E_2 (Kennedy 1979). The reason for the difference in results obtained following injection and infusion is not known. Miller and O'Morchoe (1982) found that indomethacin did not affect the decidualization in response to intrauterine-administered prostaglandin $F_{2\alpha}$.

Jonsson *et al.* (1979) have suggested that prostaglandin I_2 may be a mediator of decidualization in mice since tranylcypromine, purportedly a selective inhibitor of prostaglandin I_2 synthesis (Gryglewski *et al.* 1976), inhibits decidualization. However, the specificity of this inhibitory action of tranylcypromine has been questioned (Rajtar and

de Gaetano 1979; Buxton and Murdoch 1982), and the inhibition has not been overriden with prostaglandin I_2. Because of its chemical instability in aqueous solutions at neutral pH, it is difficult to determine the biological activity of prostaglandin I_2. When infused into the uterine lumen of indomethacin-treated rats at 1 μg/h in Tris–saline buffer, pH 9, prostaglandin I_2 was ineffective in bringing about either an increase in endometrial vascular permeability or decidualization whereas, in the same experiments under identical conditions, prostaglandin E_2 was effective (Kennedy, unpublished data).

Thus there is considerable uncertainty about the identity of the prostaglandins involved in the initiation of implantation and decidualization. Given the similarities between the early stages of implantation and the inflammatory response, it has been prostaglandins of the E, and more recently, of the I series which have been implicated in the inflammatory response (Williams and Peck 1977; Williams 1979).

Control of Uterine Sensitization

For implantation to occur, there is a strict requirement for synchronization between embryonic and endometrial development (Psychoyos 1973). Moreover, decidualization in response to artificial stimuli can only be obtained during a limited period of pregnancy, pseudopregnancy, or when the uterus has been prepared by an appropriate regimen of hormone treatments (Psychoyos 1973; Finn and Porter 1975). In addition, oestrogens in low dosages act synergistically with progesterone to sensitize the rat and mouse uterus for the decidual cell reaction (Yochim and De Feo 1963; Armstrong and King 1971; Finn and Porter 1975). That these changes in uterine sensitization might be related to the ability of the uterus to produce prostaglandins has been investigated but the results indicate that uterine prostaglandin levels in response to a standardized artificial stimulus does not provide a ready explanation for the changes in sensitization (Kennedy 1980*a*, 1980*b*; Milligan and Lytton 1983). Rather, maximum uterine sensitization corresponded with the maximum ability of the endometrium to respond to intrauterine-injected prostaglandin E_2 with increased endometrial vascular permeability (Kennedy 1980*a*, 1980*b*).

There are several possible explanations for these findings. In the non-responsive uterus, the exogenous prostaglandins may be metabolized rapidly and are therefore ineffective; metabolism of prostaglandins by sensitized and non-sensitized endometrium has not been investigated. Alternatively, increased endometrial vascular permeability may require the action of mediators in addition to prostaglandins (as has been suggested for the inflammatory response—Williams 1977; Williams and Peck 1977) and it is the production, release or action of these other mediators which determines maximum uterine sensitization. Power and Kennedy (1982) attempted unsuccessfully to override oestrogen-induced unresponsiveness by the intrauterine injection of prostaglandin E_2 combined with either histamine or bradykinin. Finally, endometrial responsiveness may be related to the properties of endometrial receptors for prostaglandins. Kennedy *et al.* (1983*a*) have recently found that an endometrial membrane preparation from sensitized rat uteri has specific, saturatable, high-affinity binding sites for E-series prostaglandins. However, although the onset of uterine sensitization is temporally correlated with the appearance of detectable concentrations of these binding sites (Kennedy *et al.* 1983*a*), no simple relationship exists between their endometrial concentrations and uterine sensitization for the decidual cell reaction (Kennedy *et al.* 1983*b*). The endometrial concentration of binding sites are controlled primarily by progesterone and they seem to be located in the stroma but not luminal epithelium (Kennedy *et al.* 1983*b*). These studies need to be extended to determine if there are receptors within the endometrium for other prostaglandins.

Mode of Action of Prostaglandins

Little is known about the mechanisms by which prostaglandins bring about increased endometrial vascular permeability and subsequent decidualization. The studies of Tobert

(1976) and Kennedy and Lukash (1982) suggest that prostaglandins are involved not only in the permeability response but also throughout the transformation of stromal cells to decidual cells. The mechanism of action as well as the types of prostaglandins may differ in these two processes.

Arguing by analogy with the inflammatory response, Kennedy and Armstrong (1981) have suggested that there may be two mediators of the endometrial vascular permeability response; one, a prostaglandin of the E or I series, may cause vasodilation; the other, possibly histamine, may increase vascular permeability. In support of this are the observations that vasodilation accompanies the endometrial permeability response to an artificial stimulus (Bitton *et al.* 1965) and, as reviewed above, that histamine may be involved in implantation.

At the cellular level, the effects of prostaglandins may be mediated by alterations in intracellular levels of adenosine 3′5′-cyclic monophosphate (cAMP). Prostaglandins of the E and I series are stimulators of cAMP synthesis in a number of cell types (Kuehl *et al.* 1976; Singhal *et al.* 1976; Goff *et al.* 1978; Omini *et al.* 1979) and artificial deciduogenic stimuli bring about a rapid increase in uterine cAMP levels (Leroy *et al.* 1974; Rankin *et al.* 1977, 1979; Kennedy 1983). The increase in uterine cAMP concentrations in response to deciduogenic stimuli is inhibited by indomethacin, suggesting that the response is prostaglandin-mediated (Rankin *et al.* 1979; Kennedy 1983). Cholera toxin, a stimulator of adenylate cyclase, is a potent inducer of endometrial vascular permeability changes in rats (Kennedy 1983) and of decidualization in rats and mice (Rankin *et al.* 1979; Kennedy 1983). Additional evidence for the involvement of cAMP in the initiation of implantation has been obtained by Dey and Hubbard (1981) who reported that the intrauterine administration of an inhibitor of adenylate cyclase reduced the implantation rate in rabbits.

The endometrial cells which respond to prostaglandins with increased cAMP synthesis are unknown. If E-series prostaglandins are the mediators, then presumably these cells are within the endometrial stroma since high-affinity binding sites for E-series prostaglandins were detected in the stroma but not epithelium (Kennedy *et al.* 1983*b*). Endometrial stroma is not a homogeneous tissue; it consists of vascular endothelium and stromal cells, as well as other cells. It would be of great interest to know if prostaglandins modify cAMP synthesis in both endothelial cells and stromal cells as this may be of importance in regulating their function.

Conclusion

The signal by which the blastocyst makes its presence known to the endometrium and brings about the initiation of implantation has not been definitively established. In the present review, evidence for four different signals has been considered. These signals are not mutually exclusive; endometrial prostaglandin production, for example, may be stimulated as a consequence of physical interaction between the blastocyst and the luminal epithelium. At present, it is not possible to exclude any of the signals with certainty. The bulk of the experimental evidence certainly suggests that prostaglandins have an obligatory role in the initiation of implantation, but little is known about the types of prostaglandins involved, their site of production or mode of action. In addition, it is very likely that the prostaglandins do not act alone, but interact with other compounds.

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