THE CONTROL OF IMPLANTATION

C. A. FINN AND L. MARTIN

*Department of Physiology, The Royal Veterinary College, London, N.W.1, and
The Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2*

The control mechanisms involved in implantation can be divided into those operating from outside the uterus and intracellular controls within the organ. The hormones of the ovary ensure that uterine preparation is synchronized with the presence of a mature blastocyst in the uterine lumen, whilst the intracellular controls regulate and integrate the changes which take place within the organ and between it and the blastocyst. A prominent feature of these changes in many species is the transformation of the connective tissue stromal cells into specialized decidual cells in which the blastocyst comes to lie, either by passing through the uterine epithelium or by degeneration of the epithelium around it. This transformation will be referred to as the decidual cell reaction (DCR).

HORMONAL CONTROL

In the majority of animals implantation takes place at a fixed interval of time after ovulation (assuming a fertile copulation) when the corpus luteum is fully formed. This is during the luteal phase of the menstrual cycle or the dioestrous phase of the oestrous cycle. Implantation can thus be considered as the culminating event of the oestrous cycle, and it follows that any investigation into the hormonal control of implantation must start by considering the pattern of hormone secretion during the oestrous or menstrual cycle. Obviously the hormones secreted during the cycle are the only ones which can be involved in normal implantation. It may be interesting to determine what the uterus is capable of achieving under experimental conditions, but ultimately the results must be related to the events actually taking place in the animal during the period before implantation. In this paper the pattern of hormone secretion, as far as it is known, is presented and followed by a discussion of the extent to which the hormones are involved in the control of implantation.

Until recently it was not possible to measure accurately the levels of ovarian hormones and much of the information on the control of implantation was deduced from indirect biological evidence, mainly from rodents. The biochemical data have, on the whole, confirmed earlier biological results.

The pattern of hormone secretion in the human menstrual cycle is shown in Text-fig. 1 (see also Somerville, 1971). A similar pattern occurs in other species, including the cow (Shemesh, Ayalon & Lindner, 1972), ewe (Cox, Mattner & Thorburn, 1971), monkey (Hotchkiss, Atkinson & Knobil, 1971), bitch and post-copulatory rat (Nimrod, Ladany & Lindner, 1972). There are three main stages. First, a period when oestrogen levels are high and virtually no progesterone is secreted. This is the pro-oestrous or follicular stage of the cycle.

TEXT-FIG. 1. Urinary oestrogen excretion and plasma progesterone levels during a normal menstrual cycle (from chart by Searle Scientific Services, High Wycombe, Bucks). Shaded areas indicate menstruation.

Second, a stage when the levels of both hormones are low immediately following ovulation and, finally, a stage when both progesterone and oestrogens are secreted. Implantation occurs towards the end of this stage, the luteal phase. It has not yet been possible to measure the secretion of the hormones during the mouse cycle but from indirect biological data the pattern shown in Text-fig. 2 has been worked out for the period preceding implantation (Finn & Martin, 1969); it follows closely that of other species. For convenience we refer to the first period of oestrogen secretion as pro-oestrous oestrogen and the second as luteal-phase oestrogen.

The next problem is the extent to which these hormonal parameters are

TEXT-FIG. 2. Pattern of ovarian hormone secretion in the mouse from indirect biological data (Finn & Martin, 1969). A = pro-oestrous oestrogen; B = luteal-phase oestrogen; C = progesterone.

involved in controlling the preparation of the uterus for implantation. Of necessity most of the discussion will centre on work using mice and rats.

To deal with the easiest problem first; it can be stated as a general rule that progesterone is required for implantation. Knowledge of the rôle of the corpus luteum and of progesterone is of very long standing (Prenant, 1898; Fraenkel & Cohn, 1901; Corner, 1928; Allen & Corner, 1929). There appears to be an absolute requirement for progesterone in the great majority of species and the dose-response curve is so steep that the relationship is almost all or none.

The rôle of the two periods of oestrogen secretion in implantation is equivocal. In 1908, Loeb showed that traumatization of the uterus of a guinea-pig at about the expected time of implantation induced in the endometrium a reaction closely resembling the reaction of the uterus to implantation. This traumatic deciduoma reaction was soon demonstrated in other rodents (e.g. rat: Selye & McKeown, 1935) and has for many years been used as a model for studying the endocrine factors in implantation in the belief that it required the same factors for sensitization of the endometrium as did implantation. It followed, therefore, that since the traumatic DCR could be induced in ovariectomized rats and mice treated with progesterone alone, no other hormone was thought to be involved in implantation. There was, however, the problem of the delayed implantation that follows a post-partum mating in rats and mice if the young are allowed to suckle (Lataste, 1891; Kirkham, 1916). In this condition the corpora lutea, histologically at least, appear to be functional and the uterus progestational but the blastocysts do not implant. However, deciduomata can be induced by traumatizing the uterus, confirming that progesterone is being secreted but suggesting that something necessary for implantation, possibly a hormone, is lacking.

The suspicion that another hormone was involved was strengthened when Krehbiel (1941) showed that implantation could be precipitated in lactating rats by the injection of a small dose of oestradiol. Similar findings were later made in the mouse (Bloch, 1943; Whitten, 1958). These results led to the hypothesis that a small quantity of oestrogen is necessary for implantation and its absence is the cause of the delayed implantation of lactation in rodents. Later experiments have provided more direct evidence for the rôle of oestrogen in normal implantation in rats (Canivenc, Laffargue & Meyer, 1956; Cochrane & Meyer, 1957). Briefly, these experiments showed that if pregnant rats were ovariectomized before implantation and given exogenous progesterone, implantation depended on the time of ovariectomy. If the operation was performed after a certain critical time relative to ovulation, the blastocysts implanted normally; rats ovariectomized before this time, however, would go into a condition of delayed implantation, in which the blastocysts remained quiescent but could be induced to implant by a small dose of oestrogen. This work has been followed by many confirmatory experiments (see Nutting & Meyer, 1963), notably those involving the transfer of blastocysts to hormone-treated ovariectomized animals (Psychoyos, 1961a; Humphrey, 1969), which demonstrate that in rats and mice (Bloch, 1959; Smith & Biggers, 1968) implantation normally involves a period of progesterone secretion and a surge of oestrogen (Psychoyos, 1967).

At this point one might enquire why it is that the traumatic deciduoma can be induced in the uteri of animals not given oestrogen. A likely answer to this problem came when it was shown that the intrauterine injection of oil was a very good decidual stimulus in pseudopregnant rats (Finn & Keen, 1962) and mice (Finn & Hinchliffe, 1964) but, unlike trauma, it was ineffective in animals treated with progesterone only (Finn, 1965). Like the blastocyst, but unlike trauma, oil acts on the surface of the uterine epithelial cells so it is possible that it is here that the luteal oestrogen acts. In other words oestrogen is necessary for sensitization of the epithelial cell surface so that it can receive the stimulus of the blastocyst or oil, while trauma is independent of oestrogen because it bypasses this initial reaction and acts directly on the stromal cells (Finn, 1965).

The evidence is now strong that under normal conditions luteal oestrogen plays a rôle in the control of implantation in rats and mice (see Marcus & Shelesnyak, 1970, for review). There is some disagreement about whether the oestrogen is secreted as a short surge (Shelesnyak, 1960) or as a more continuous secretion (Yochim & DeFeo, 1963; Finn & Martin, 1969). This point is not of great importance and will in any case be answered when accurate measurements of the hormones are available.

Similar experiments to those described have also been performed on other animals, for example rabbits (Chambon, 1949; Hafez & Pincus, 1956), guinea-pigs (Deanesly, 1960) and hamsters (Orsini & Meyer, 1959) but have not pointed to an essential rôle for luteal oestrogen in implantation. Possibly in these and maybe other species oestrogen is not secreted during the luteal phase or alternatively is secreted but plays only a minor part, or none, in implantation, or perhaps under the conditions of the experiment other factors are compensating for the lack of oestrogen.

The final problem is the rôle if any, of pro-oestrous oestrogen. Undoubtedly oestrogen is secreted in most species during pro-oestrus and has many effects outside the uterus (e.g. vaginal cornification, sex behaviour, ovum transport, etc.). The question of concern in this paper is whether it influences the uterine preparation for implantation. The priming effect of oestrogen as preparation for the action of progesterone has been known for a long time (Weichert, 1928), especially in the rabbit, in which the characteristic response to progesterone is not elicited unless the animal has previously been primed with oestrogen (Clauberg, 1930). In the mouse there is also good evidence that priming oestrogen can alter considerably the subsequent response of the uterus to progesterone (Finn & Martin, 1970).

Some experiments on the control of cell division in the mouse uterus bear upon this point. During early pregnancy (Text-fig. 3) there is a sudden switch in the pattern of mitosis from being predominantly glandular on Day 3 to almost entirely stromal on Day 4 (Finn & Martin, 1967). The control of this change was of considerable interest because it was thought to be important in preparing the stromal cells for decidualization, perhaps being in the nature of a quantal mitosis. Briefly, it was found that the only way to get the rapid change from glandular to stromal mitosis in ovariectomized mice was by administering priming oestrogen as well as progesterone and luteal oestrogen (Finn & Martin, 1970). The distribution of mitosis in the uterus of ovariectomized mice killed on

TEXT-FIG. 3. Histograms showing the switch-over from epithelial to stromal mitosis in the uterus between Days 3 and 4 of pregnancy in the mouse. Solid column = luminal epithelium; open column = glandular epithelium; shaded column = stromal epithelium.

succeeding days after the administration of three daily injections of 100 ng oestradiol is shown in Text-fig. 4. A large number of gland cells were undergoing mitosis on the fourth day after the end of priming. In terms of pregnancy in the mouse this is equivalent to Day 3 of pregnancy (assuming Day 1, the day of mating, is equivalent to the last day of full vaginal cornification). It is possible therefore to explain the glandular mitosis on Day 3 as being caused by the oestrogen secreted before oestrus.

E E E - K
 E E E - - K
 E E E - - - K
 E E E - - - K
 E E E - - - K
 - - - - - - K

TEXT-FIG. 4. Histogram showing the pattern of mitosis in the uterus on successive days after 3 days priming with 100 ng oestradiol-17 β (EEE). K = day of autopsy (2 hr after the administration of colcemid). Shading as in Text-fig. 3. (Data from Finn & Martin, 1970.)

Text-figure 5 shows the effect on endometrial mitosis of administering a single injection of progesterone, or progesterone and oestradiol, on various days after priming and killing the animals 24 hr later (2 hr after colcemid). In the unprimed animals progesterone, with or without oestradiol, stimulated mainly luminal mitosis but no stromal mitosis. This response was, however, completely changed by priming, so that if the injection was given on Day 4

TEXT-FIG. 5. Mitosis following the administration of a single dose of (a) 1 mg progesterone (P) or (b) of 1 mg progesterone plus 20 ng oestradiol (Pe), on various days after priming. The mice were killed 24 hr later, 2 hr after administration of colcemid. Shading as in Text-fig. 3. (Data from Finn & Martin, 1970.)

after priming there was a very big response in the stroma, especially if both hormones were given, although oestrogen alone will also give quite a large stromal response (Finn & Martin, 1970). This experiment demonstrates that it is possible to obtain three basically different responses to the administration of oestrogen by varying the pattern of administration. If this is true of cell division it is probably also true of other morphological or biochemical responses

of the uterus and cautions against simple statements about the action of oestrogen.

It can be seen from Text-figs 4 and 5 that, by priming and then giving a dose of progesterone plus oestradiol 4 days later, it is possible to get the switch from glandular to stromal mitosis similar to that found in early pregnancy. It is possible to get stromal mitosis without priming simply by giving progesterone for at least 3 days and then a dose of oestradiol (Martin & Finn, 1968, 1970), but the only way of getting the rapid changeover is first to prime the animals with oestrogen. This point is stressed to emphasize the care needed in extrapolating from experiments to the physiological situation.

TEXT-FIG. 6. Decidual response to an intrauterine injection of arachis oil given on the 3rd day of daily treatment with 500 μ g progesterone plus 10 ng oestradiol. In all except the last group (NP) the animals had been previously primed with three daily injections of 100 ng oestradiol and an interval of 1 to 5 days (as indicated) left before the start of the progesterone and oestrogen injections. (Data from Finn & Martin, 1972.)

These experiments have shown that pro-oestrous oestrogen is involved in the morphological changes in the uterus in preparation for implantation, but this does not necessarily mean that it is essential for sensitization of the uterus. To obtain some information on this, experiments were performed on ovariectomized mice, using the oil-induced DCR (Text-fig. 6).

The animals were primed for 3 days as before and then, starting on various days after the last priming injection, three daily injections of progesterone plus oestradiol were given. On the 3rd day arachis oil was injected into the uterine lumen and the decidual response was assessed 2 days later.

Without priming there was a small decidual response but it was very much less than that in animals which had been primed, especially if the interval after priming was 3 days. These results suggest that pro-oestrous oestrogen is involved in the development of maximal sensitivity to decidual stimuli, although it must be admitted that some decidualization is possible without priming. It has also been shown that implantation of transferred blastocysts is possible in unprimed animals (Humphrey, 1969), so that although pro-oestrous oestrogen may normally play a part in the development of full sensitivity it is certainly not essential for implantation.

To summarize, there is good evidence that the secretion of the ovarian hormones throughout the cycle is patterned to produce maximum uterine sensitivity at the time when the blastocyst is ready to attach, but it is not possible to say that any one of the parameters is essential in all species.

INTRACELLULAR CONTROL

The hormonal controls just discussed ensure maximum endometrial sensitivity when the mature blastocyst is present in the uterus. A reaction then takes place between the trophoblast and the uterine epithelium so that the endometrium is stimulated to undergo a chain of reactions leading to the formation of the placenta (Krehbiel, 1937; Finn & Hinchliffe, 1964). We know little about the nature of the stimulus given by the blastocyst. As already mentioned it can be mimicked by several artificial stimuli, for example air (Orsini, 1963) or oil (Finn & Keen, 1962), and there have been suggestions that CO₂ in the air may be involved (Hetherington, 1968; McLaren, 1970) or that the stimulus may be a contact reaction between the two surfaces, in which the oil is able to take part (Finn & Hinchliffe, 1964).

Once started, the changes taking place in the endometrium proceed in an orderly fashion, although once again little is known of the control mechanisms involved. The response is dependent on progesterone and if the supply of this hormone is cut off the reaction stops and regression takes place. Provided sufficient progesterone is available, however, the endometrial response appears to be independent of the level or duration of hormone secretion and follows a predetermined path culminating in the formation of the placenta or, in the case of the artificial reaction, growth of the decidua to a maximum size and then regression (Atkinson, 1944). Progesterone thus acts in a permissive rather than a directive manner, and the mechanisms responsible for directing the morphogenic changes must lie within the cells.

EXPLANATION OF PLATE 1

FIG. 1. Blastocyst implanted in the uterine stroma 48 hr after the initiation of implantation by oestradiol. (From Finn & Bredl, 1973.)

FIG. 2. Blastocyst at the same time after initiation of implantation as in Pl. 1, Fig. 1 but in the uterus of an animal treated with actinomycin D. The epithelium round the blastocyst has become stratified except at one point where the trophoblast appears to be penetrating it. (From Finn & Bredl, 1973.)

PLATE 1

(Facing p. 202)

One would expect the control to be exerted, at least partly, through information coded on the nuclear DNA, via the transcription of messenger RNA and the synthesis of enzymes. Enzyme changes have been recorded in the uterus during implantation, perhaps the most obvious being the induction of alkaline phosphatase in the decidualizing stroma (Finn & Hinchliffe, 1964).

If such control exists it should be possible to interfere selectively with those parts of the reaction which are dependent on transcription by administering the drug, actinomycin D, which blocks transcription. Glasser (1965) and Burin & Sartor (1965) have shown that the drug will inhibit the traumatic DCR and implantation in rats. More detailed study of the reaction in mice (Finn & Martin, 1972) showed that actinomycin D, given as a single dose before the sensitizing dose of oestradiol, did not prevent the pontamine blue reaction (Psychoyos, 1961b) or stromal oedema in response to an oil decidual stimulus, but the transformation of stromal cells into decidual cells, and the induction of alkaline phosphatase, were prevented and the uterus remained in the oedema stage. After a delay of about 30 hr, provided the administration of progesterone was continued, decidualization started and continued normally.

From this it appears that the sensitization of the uterus by oestrogen does not involve transcription. This is rather surprising since other actions of oestrogen are sensitive to actinomycin D and there is a lot of evidence that RNA is involved in the action of this hormone. We have ourselves shown that oestrogen-induced mitosis is completely blocked by the drug. The results also suggest that the initial stimulus to the uterus is not dependent on transcription. What was most surprising was that once the reaction had been triggered it could be delayed for a period of about 30 hr and then proceed when the effect of the drug had worn off.

More recently, a study has been made of what happens when blastocysts from mice in delayed implantation are transferred to sensitized uteri which have been treated with actinomycin D (Finn & Bredl, 1973). These experiments set out to answer two main questions: would the stimulus to the blastocyst be affected and, if the blastocyst were still activated, would the trophoblast pass through the epithelium? There has been some dispute about whether the degeneration of the uterine epithelium is due to invasion by the trophoblast or to inherent cell death (see Blandau, 1961).

As with the oil stimulus, the uterus, in spite of actinomycin D, reacted to the blastocyst and there was a pontamine blue reaction and oedema in the stroma. Adhesion between the trophoblast and the epithelial surface also occurs normally (Pollard, Bredl & Finn, 1973). The blastocyst appeared to be activated normally; not only did it increase in size but many cells undergoing mitosis could be seen in the embryonic knob and trophoblast. As before, decidual transformation of the endometrial stroma was delayed. This had the effect of putting blastocyst development out of phase with uterine morphogenesis. The normal degeneration of the uterine epithelium also failed to occur at the normal time. In control blastocysts 48 hr after transfer, the epithelium is completely lost and the trophoblast is in contact with the decidual cells (Pl. 1, Fig. 1). On the other hand, blastocysts taken from an animal treated with actinomycin D and killed 48 hr after transfer (Pl. 1, Fig. 2) show that the epithelium, far from degenerat-

ing, is building up around the blastocyst, except at one point where the trophoblast appears to be having some success in penetrating the epithelium. In all our experiments the retention of the epithelium was an invariable consequence of giving actinomycin D, which suggests that degeneration of the epithelium is controlled from within the cells through the genome. Similar examples of what is sometimes called 'programmed cell death' occur during embryogenesis, for example, in the degeneration of the Müllerian or Wolffian ducts during the development of the reproductive tract or in the resorption of the tails of tadpoles.

Regarding the lack of effect on the activation of the blastocyst (see Weitlauf, 1974) we need say very little except to point out that it does not appear to depend on the transcription of DNA in the uterine cells, as one would expect if some substances were being synthesized to stimulate the blastocyst. As far as these experiments go they suggest that the earliest parts of the implantation reaction do not directly involve transcriptional control.

ACKNOWLEDGMENTS

C.A.F. was supported financially by grants from the Medical Research Council and the Wellcome Trust during the course of much of the work described in this review. We also acknowledge the support of Miss Gillian Blair in the preparation of the manuscript.

REFERENCES

- ALLEN, W. M. & CORNER, G. W. (1929) Physiology of the corpus luteum. Normal growth and implantation of embryos after very early ablation of the ovaries under the influence of extracts of corpora lutea. *Am. J. Physiol.* **88**, 340.
- ATKINSON, W. B. (1944) The persistence of decidualomata in the mouse. *Anat. Rec.* **88**, 271.
- BLANDAU, R. J. (1961) Biology of eggs and implantation. In *Sex and Internal Secretions*, vol. 2, pp. 797-882. Ed. W. C. Young. Baillière, Tindall & Cox, London.
- BLOCH, S. (1943) Ueber die Wirkung Milchdeusekretion auf die Nidation bei der Maus. *Schweiz. med. Wschr.* **73**, 245.
- BLOCH, S. (1959) Untersuchungen über die hormonalen Grundlagen der Nidation. *Gynaecologia*, **148**, 157.
- BURIN, P. & SARTOR, P. (1965) Inhibition de la reaction déciduale par l'actinomycine D. *C. r. Séanc. Soc. Biol.* **159**, 141.
- CANIVENC, R., LAFFARGUE, M. & MEYER, G. (1956) Nidation retardées chez la ratte castrée et injectée de progestérone: influence du moment de la castration sur la chronologie de l'ovo-implantation. *C. r. Séanc. Soc. Biol.* **150**, 2208.
- CHAMBON, Y. (1949) Besoins endocriniens qualitatifs et quantitatifs de l'ovoimplantation chez la lapine. *C. r. Séanc. Soc. Biol.* **143**, 1172.
- CLAUBERG, C. (1930) Sexual hormones im besanderen des hormons des Corpus Luteum. *Zentbl. Gynäk.* **54**, 2757.
- COCHRANE, R. L. & MEYER, R. K. (1957) Delayed nidation in the rat induced by progesterone. *Proc. Soc. exp. Biol. Med.* **96**, 155.
- CORNER, G. W. (1928) Physiology of the corpus luteum. The effect of very early ablation of the corpus luteum upon embryos and uterus. *Am. J. Physiol.* **86**, 74.
- COX, R. I., MATTNER, P. E. & THORBURN, G. D. (1971) Changes in ovarian secretion of oestradiol-17 β around oestrus in sheep. *J. Endocr.* **49**, 345.
- DEANESLY, R. (1960) Implantation and early pregnancy in ovariectomized guinea-pigs. *J. Reprod. Fert.* **1**, 242.
- FINN, C. A. (1965) Oestrogen and the decidual cell reaction of implantation in mice. *J. Endocr.* **32**, 223.
- FINN, C. A. & BREDL, J. C. S. (1973) Studies on the development of the implantation reaction in the mouse uterus: influence of actinomycin D. *J. Reprod. Fert.* **34**, 247.

- FINN, C. A. & HINCHLIFFE, J. R. (1964) The reaction of the mouse uterus during implantation and decidual formation as demonstrated by changes in the distribution of alkaline phosphatase. *J. Reprod. Fert.* **8**, 331.
- FINN, C. A. & KEEN, P. M. (1962) Studies on decidual formation in the rat. *J. Reprod. Fert.* **4**, 215.
- FINN, C. A. & MARTIN, L. (1967) Patterns of cell division in the mouse uterus during early pregnancy. *J. Endocr.* **39**, 593.
- FINN, C. A. & MARTIN, L. (1969) Hormone secretion during early pregnancy in the mouse. *J. Endocr.* **45**, 57.
- FINN, C. A. & MARTIN, L. (1970) The role of oestrogen secreted before oestrus in the preparation of the uterus for implantation in the mouse. *J. Endocr.* **47**, 431.
- FINN, C. A. & MARTIN, L. (1972) Temporary interruption of the morphogenesis of decidual formation in the mouse uterus by actinomycin D. *J. Reprod. Fert.* **31**, 353.
- FRAENKEL, L. & COHN, F. (1901) Experimentelle Untersuchung über den Einfluss des Corpus Luteum und die Insertion des Eies. *Anat. Anz.* **20**, 294.
- GLASSER, S. R. (1965) Biochemical studies on a cellular receptor mechanism for uterine sensitivity. *Excerpta Med. Int. Congr. Series*, **99**, 335.
- HAFEZ, E. S. E. & PINCUS, G. (1956) Hormonal requirements of implantation in the rabbit. *Proc. Soc. exp. Biol. Med.* **91**, 531.
- HETHERINGTON, C. M. (1968) Induction of decidual formation in the mouse by carbon dioxide. *Nature, Lond.* **219**, 863.
- HOTCHKISS, J., ATKINSON, L. E. & KNOBIL, E. (1971) Time course of serum and luteinizing hormone concentrations during the menstrual cycle of the rhesus monkey. *Endocrinology*, **89**, 177.
- HUMPHREY, K. W. (1969) Induction of implantation of blastocysts transferred to ovariectomized mice. *J. Endocr.* **44**, 299.
- KIRKHAM, W. B. (1916) The prolonged gestation period in suckling mice. *Anat. Rec.* **11**, 31.
- KREHBIEL, R. H. (1937) Cytological studies of the decidual reaction in the rat during pregnancy and in the production of decidual formation. *Physiol. Zool.* **10**, 212.
- KREHBIEL, R. H. (1941) The effects of theelin on delayed implantation in the pregnant lactating rat. *Anat. Rec.* **81**, 381.
- LATASTE, F. (1891) Des variations de durée de la gestation chez les mammifères et des circonstances qui déterminent ces variations. Théorie de la gestation retardée. *C. r. Séanc. Soc. Biol.* **43**, 21.
- LOEB, L. (1908) The experimental production of the maternal placenta and the formation of the corpus luteum. *J. Am. med. Ass.* **50**, 1897.
- McLAREN, A. (1970) Early embryo-endometrial relationships. In *Ovo-implantation, Human Gonadotrophins and Prolactin*, pp. 18–37. Eds. P. O. Hubinont, F. Leroy, C. Robyn and P. Leleux. Karger, Basel.
- MARCUS, G. J. & SHELESNYAK, M. C. (1970) Steroids in nidation. In *Advances in Steroid Biochemistry and Pharmacology*, Vol. 2, pp. 373–440. Ed. M. H. Briggs. Academic Press, London and New York.
- MARTIN, L. & FINN, C. A. (1968) Hormonal regulation of cell division in epithelial and connective tissues of the mouse uterus. *J. Endocr.* **41**, 363.
- MARTIN, L. & FINN, C. A. (1970) Interactions of oestradiol and progestins in the mouse uterus. *J. Endocr.* **48**, 109.
- NIMROD (ZMIGROD) A., LADANY, S. & LINDNER, H. R. (1972) Perinidatory ovarian oestrogen secretion in the pregnant rat, determined by gas chromatography with electron capture detection. *J. Endocr.* **53**, 249.
- NUTTING, E. F. & MEYER, R. K. (1963) Implantation delay, nidation and embryonal survival in rats treated with ovarian hormones. In *Delayed Implantation*, pp. 233–251. Ed. A. G. Enders. University of Chicago Press.
- ORSINI, M. W. (1963) Induction of decidual formation in hamsters and rats by injection of air. *J. Endocr.* **28**, 119.
- ORSINI, M. W. & MEYER, R. K. (1959) Implantation of the castrate hamster in the absence of exogenous estrogen. *Anat. Rec.* **134**, 619, Abstr.
- POLLARD, R. M., BREDL, J. C. S. & FINN, C. A. (1973) The effect of actinomycin D on the attachment reaction of implantation in mice. *J. Reprod. Fert.* **33**, 343.
- PRENANT, L. A. (1898) De la valeur morphologique du corps jaune, son action physiologique et thérapeutique possible. *Rev. gén. Sci. pur. appl.* **9**, 646.
- PSYCHOVOS, A. (1961a) Nouvelles recherches sur l'ovoimplantation. *C. r. hebd. Séanc. Acad. Sci., Paris*, **252**, 2306.
- PSYCHOVOS, A. (1961b) Perméabilité capillaire et décidualisation utérine. *C. r. hebd. Séanc. Acad. Sci., Paris*, **252**, 1515.
- PSYCHOVOS, A. (1967) The hormonal interplay controlling egg-implantation in the rat. *Adv. Reprod. Physiol.* **2**, 257.

- SELYE, H. & McKEOWN, T. (1935) Studies on the physiology of the internal placenta of the rat. *Proc. R. Soc. B*, **119**, 1.
- SHELESNYAK, M. C. (1960) Nidation of the fertilized ovum. *Endeavour*, **19**, 81.
- SHEMESH, M., AYALON, N. & LINDNER, H. R. (1972) Oestradiol levels in the peripheral blood of cows during the oestrous cycle. *J. Endocr.* **55**, 73.
- SMITH, D. M. & BIGGERS, J. D. (1968) The oestrogen requirement for implantation and the effect of its dose on the implantation response in the mouse. *J. Endocr.* **41**, 1.
- SOMERVILLE, B. W. (1971) Daily variations in plasma levels of progesterone and oestradiol throughout the menstrual cycle. *Am. J. Obstet. Gynec.* **111**, 419.
- WEICHERT, C. K. (1928) Production of placentomata in normal and ovariectomized guinea pigs and albino rats. *Proc. Soc. exp. Biol. Med.* **25**, 490.
- WEITLAUF, H. M. (1974) Metabolic changes in the blastocysts of mice and rats during delayed implantation. *J. Reprod. Fert.* **39**, 213.
- WHITTEN, W. K. (1958) Endocrine studies on delayed implantation in lactating mice: role of the pituitary in implantation. *J. Endocr.* **16**, 435.
- YOCHIM, J. M. & DEFEO, V. J. (1963) Hormonal control of the onset, magnitude and duration of uterine sensitivity in the rat by steroid hormones of the ovary. *Endocrinology*, **72**, 317.