Oestrogen-dependence of decidualization in some Myomorph rodents: basic endocrine mechanisms and their biological significance

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In several species of Myomorph rodents implantation can be delayed by a concurrent lactation or by various experimental procedures. Furthermore, it has been shown that this delay is due to a postponement of the secretion of « nidatory » oestrogen during the luteal phase. These phenomena — lactational delay and oestrogen-dependence of implantation — have been repeatedly discussed in several review papers (COURRIER, 1945 ; 1950 ; COURRIER & BACLESSE, 1955 ; CANIVENC & MAYER, 1955 ; CHAMBON, 1958 ; 1966 ; MAYER, 1959*a* ; 1959*b* ; 1960*a* ; 1960*b* ; 1963 ; SHELESNYAK, 1960 ; SHELESNYAK & KRAICER, 1960 ; 1963 ; NUTTING & MEYER, 1963 ; PSYCHOYOS, 1965 ; 1967*a* ; 1967*b* ; 1968 ; DEANESLY, 1966 ; DE FEO, 1967).

The present paper represents an attempt to elucidate some points which do not emerge clearly from previous discussions. Therefore, we shall give only a brief summary of the data concerning those interactions between progestogen and oestrogen which lead to nidation (*). No mention will be made of the hormonal conditions prevailing after nidation or successful induction of decidualization by means of artificial stimuli. In fact, it appears clearly that both progestogens and oestrogens are necessary in order to establish optimal conditions in the latter phase. The discussion will be limited to the hormonal balance during the period between oestrus and implantation, without reference to the cellular and molecular changes triggered or permitted by the hormones. Finally, delayed implantation and oestrogen-dependence of implantation will be discussed in relationship to the biological characteristics of the species involved.

(*) For this reason, original references will be used sparingly in this and the two following sections, which represent a brief excerpt of the reviews mentioned in the opening paragraph.

Delayed implantation. — Delayed implantation takes place in several mammalian species belonging to different taxonomic groups. However, the available data indicate that a variety of physiological mechanisms is involved (CANIVENC, 1960 ; ENDERS, 1963 ; DEANESLY, 1966). We are interested here in the lactational delay observed in Myomorph rodents when animals ovulate and mate in the *post-partum* period. Although this has been observed in several species, nearly all systematic analyses have dealt with laboratory rats and mice.

In the 70 years which have elapsed since the first publications of the French biologist LATASTE (quoted by MAYER, 1959*b*), several facts have been firmly established. Implantation can be delayed not only by lactation, but also by means of various experimental procedures : *a*) early ovariectomy followed by progesterone treatment, or transfer of blastocysts into ovariectomized, progesterone-treated animals ; *b*) hypophysectomy followed by treatment with prolactin or progesterone, or by autotrasplantation of the pituitary ; *c*) administration of small doses of anti-LH serum (larger doses interrupt pregnancy) ; *d*) administration of synthetic progestogens, presumably blocking the release of FSH and/or LH ; *e*) treatment with neuroleptics such as phenothiazine derivatives and Rauwolfia alkaloids ; *f*) pregnancy in prepuberal animals in which ovulation and mating are provoked by gonadotrophins ; *g*) various kinds of stress.

There is ample evidence that lactation and various experimental procedures act through a single mechanism, i.e., the lack of an oestrogen phase during progestation, distinct from that of the previous oestrus period. The procedures listed above show how this result can be obtained in widely differing conditions, ranging from a direct control of the steroid hormones administered to ovariectomized animals to changes of the gonadotrophic activity of the pituitary. In fact, pituitary autotransplantation or treatment with tranquilizers lead to a predominance of prolactin secretion and a reduction of other gonadotrophic activities.

Three points must be stressed here. First, some of the data presented by PSYCHOYOS (1962) and by SMITHBERG & RUNNER (1956 ; 1960) indicate that there exist borderline conditions in which both oestrogens and progestogens are present in small amounts, and implantation does not take place. It seems that, in these conditions, either the administration of additional oestrogen or the administration of additional progestogen can lead to implantation. Second, it appears that blastocysts can survive for a certain period of time in the absence of both progestogens and oestrogens (CANIVENC & LAFFARGUE, 1957 ; WEITLAUF & GREENWALD, 1968). Obviously, in this case the administration of oestrogen alone cannot lead to implantation. Finally, it has been shown that blastocysts exposed *in utero* to the action of oestrogen and then transferred to the uterus of progesterone-treated

animals can implant only if the acceptor is also treated with oestrogen (PSYCHOYOS, 1961). This indicates that the exposure of the progestational endometrium to oestrogen is the critical factor for implantation, or at least that it is much more important than the exposure of the blastocysts.

The sequence of changes in uterine sensitivity. — The attempts to reconstruct endocrine sequences have shown that interactions between different hormones can take place according to characteristic diphasic patterns. For example, the administration of progesterone to oestrogen-primed female guinea-pigs leads to a marked facilitation of oestrus behaviour, followed by a prolonged period of refractoriness (ZUCKER, 1966 ; 1968 ; ZUCKER & GOY, 1967) (*).

An analogous model is valid in the case of oestrogen-progestogen interactions during progravidity in the rat, except that the relative roles of the two hormones are reversed. The development of a high level of endometrial sensitivity to deciduogenic stimuli has been shown to depend on three distinct phenomena : *a*) oestrogen priming during the follicular phase ending at ovulation ; *b*) exposure to progestogen ; *c*) additional exposure to oestrogen. Furthermore, the marked increase in sensitivity provoked by the additional oestrogen is followed by a state of complete refractoriness. On the other hand, a prolonged period of low-level sensitivity to deciduogenic stimuli can be obtained with the administration of progesterone to ovariectomized animals. It is accepted that this phenomenon explains the extended low-level sensitivity observed in lactating animals. This picture must be completed by taking into account another well-known limiting condition — i.e., a high sensitivity level cannot be obtained if the interval between the end of the follicular phase and the application of deciduogenic stimuli is too short.

It appears now that this general model can explain a host of controversial data, provided that the following facts are taken into account. First, the effectiveness of deciduogenic stimuli, both natural and artificial, is far from being uniform (see, e.g., KRAICER & SHELESNYAK, 1958 ; 1959 ; DE FEO, 1963*b* ; FINN, 1965). Certain types of stimuli, e.g., trauma, can induce decidualization when the level of sensitivity is well below that required for ovoimplantation (**). On the other hand more selective stimuli, such as the histamine-releasing and antihistamine compound pyrathiazine (KRAICER

(*) The above data are given as a representative example of a diphasic sequence in a different area of endocrine physiology. It should be emphasized, however, that a phase of refractoriness after the peak of receptivity induced by progesterone has not been found in Myomorph species (rat : WHALEN & NAKAYAMA, 1965 ; ZUCKER, 1967 ; mongolian gerbil : KUEHN & ZUCKER, 1968).

(**) To some extent, this lack of specificity can be exploited for the quantitative estimation of sensitivity levels, ranging from those which do not allow implantation to those observed for a brief period of time upon completion of an optimal hormonal sequence.

& Shelesnyak, 1958 ; 1959 ; Shelesnyak & Kraicer, 1961), tend to yield all-or-none results, depending on whether or not there exists a level of sensitivity compatible with nidation.

Another controversy concerns the double dissociation between the results of experiments on implantation and those of experiments using artificial deciduogenic stimuli. Obviously, since the effectiveness of deciduogenic stimuli varies, it is easy to understand why certain hormonal states allow decidualization, but not implantation. Viceversa, the characteristic diphasic pattern provoked by an optimal hormonal sequence accounts for the opposite type of dissociation. If blastocysts are present in the uterus, the process of implantation is set in motion if, and when, appropriate conditions are created. No such guarantee operates when the deciduogenic stimulus must be applicated by the experimenter. Even if optimal conditions are created, there is a high risk of missing the peak of maximal sensitivity.

The fact that a low-level endometrial sensitivity in the absence of oestrogen can have a long duration, while a high-level sensitivity lasts for a short period of time, also explains the data on « successive deciduomata » (*déciduomes successifs* of the French authors) (see, e.g. : Peckham & Greene, 1947 ; Kehl & Douard, 1951). These results can be summarized as follows : more than one generation of deciduomata can be obtained during a given luteal phase (natural or artificial) if oestrogen is absent, while only one generation can be obtained if oestrogen is secreted or administered (*).

All the data that we have mentioned and particularly the development of endometrial sensitivity at various intervals after the end of the follicular phase, can well be explained by the « oestrogen surge » model proposed by Shelesnyak (Johnson & Shelesnyak, 1958 ; Shelesnyak, 1959 ; 1960 ; Shelesnyak & Kraicer, 1960 ; 1963 ; Shelesnyak, Kraicer & Zeilmaker, 1963). This model postulates a total or near total interruption of oestrogen secretion after the end of the follicular phase, and a resumption of such secretion at a time which depends from the physiological conditions of the animals.

The next section will discuss some data on experimental decidualization which are in general agreement with this interpretation, but still leave one point undecided. These data confirm that *the reintroduction of oestrogen on the day before the application of deciduogenic stimuli can lead to a high sensitivity to these stimuli at various times after the end of the follicular phase, provided that a minimum interval is respected. On the other hand, when a parti-*

(*) One understandable exception to this rule is represented by the obtention of asynchronous implantations in progesterone-treated rats by means of local (intrauterine) oestrogen treatments. In this case, the untreated horn does not undergo the diphasic change in sensitivity. The renewed administration of oestrogen at a later time allows implantation to take place in areas which have not completed the sequence up to the point of refractoriness.

cular temporal relationship is used, various patterns of oestrogen secretion can lead to a high level of sensitivity on day 4, but not at a later time.

Interactions between type of oestrogen treatment and temporal factors. YOCHIM & DE FEO (1963) have shown that the daily administration of 2 mg of progesterone and 1 μg of oestrone, started at the time of ovariectomy on the day after vaginal keratinization, allowed the development of a high sensitivity to deciduogenic stimuli on day 4 of the treatment. Traumatization on day 3 was much less effective, while traumatization on day 5 was totally ineffective. YOCHIM & DE FEO (1963) postulated at that time that the high sensitivity found on day 4 after a continuous oestrogen treatment made it unnecessary to postulate an oestrogen surge. The same authors found that, when the uteri were traumatized on day 5 or later, treatments with oestrogen for more than one day before the application of the stimulus prevented decidualization (YOCHIM & DE FEO, 1963; DE FEO, 1967). Obviously, this finding excluded any « continuous oestrogen secretion hypothesis » as a possible explanation for a delayed peak of sensitivity, e.g., during lactation.

This problem was reexamined in our laboratory in investigations on the interactions between various types of pretrauma oestrogen treatments and temporal factors (ROSSI CARTONI & BIGNAMI, 1966). Some critical data are reported in Fig. 1. The black bar to the left of the graph shows the average weight of decidualized uterine horns in intact rats traumatized on day 4 of pseudopregnancy and sacrificed 96 hours later (*). The other bars regard groups of animals ovariectomized about 2 weeks before the beginning of the hormonal treatments, which are indicated in Table 1. All animals received the same type of priming and the same oestrogen-progesterone combination from traumatization to autopsy.

The central group of bars (B) refers to a set of temporal relationships similar to that existing in intact pseudopregnant animals when the period of maximal sensitivity is exploited. In fact, progesterone administration was started on the day after the first appearance of complete vaginal keratinization (**), and the uteri were traumatized on the 4th day of the artificial luteal phase. Clearly, either a continuous oestrogen treatment during the artificial luteal phase (with equal doses or increasing doses) or a single

(*) Day 0 = Day of vaginal keratinization.
Day 1 = First day with leucocyte smear.

(**) When dealing with a long-acting oestrogen such as oestradiol benzoate it is necessary to speak in terms of « first appearance of complete vaginal keratinization », rather than in terms of « vaginal keratinization » *per se*. In the absence of a subsequent progesterone treatment two injection of 0.75 μg of oestradiol benzoate can induce a long-lasting vaginal oestrus in ovariectomized animals. In the case of the groups shown in Fig. 1, the keratinization was terminated after different average time intervals, depending on the day in which the progesterone treatment was started.

treatment 18 hours before traumatization induced a high level of sensitivity to the deciduogenic stimulus. The bars to the left (A) correspond to groups of animals traumatized too early, i. e., 72 hours after the first appearance of vaginal keratinization. These groups showed a uniform lack of sensitivity. The bars to the right (C) correspond to an interval of about

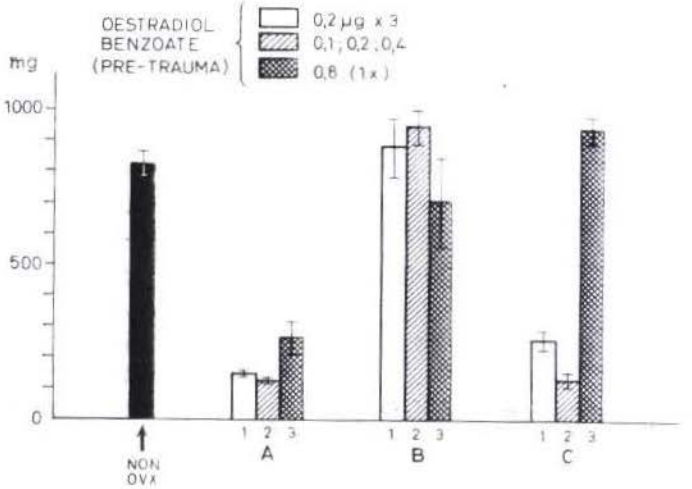

Fig. 1. — Decidualization in ovariectomized rats: interactions between I) Various types of pre-trauma oestrogen treatment (1, 2, 3); and II) Various temporal relationships between follicular phase, luteal phase, and pretrauma oestrogen treatment (A, B, C). For the schedules of hormonal treatment see Table 1. The bars give the mean weight of traumatized horns (in mg) ± SE. The black bar to the left (Non ovx) corresponds to a group of intact pseudopregnant rats traumatized on Day 4 and sacrificed on Day 8. The other bars correspond to groups of ovariectomized animals. For a description of the results see text. (Modified from Table 1 in Rossi Cartoni & Bignami, 1966).

120 hours between the first appearance of vaginal keratinization and traumatization. No decidualization was found in the two groups with continuous oestrogen treatment, while animals with a single treatment before traumatization were maximally sensitive. The latter result was confirmed by other experiments reported in the same paper (Table 2 in Rossi Cartoni & Bignami, 1966). Either in the absence of a previous follicular phase, or with an interval between follicular phase and traumatization longer than that of condition B illustrated above, only single oestrogen treatments on the day before traumatization allowed the development of a high endometrial sensitivity.

The fact that a continuous pre-trauma oestrogen treatment allows decidualization, but only with a particular temporal relationship, became even more evident when oestradiol and oestradiol benzoate were compared (De Luca, 1969).

TABLE 1.

Schedules of hormonal treatment used in the experiments described in Fig. 1.

Oe = Oestradiol benzoate (μg); P = Progesterone (mg); Tr = Traumatization day; N = Number of rats; (a) = vaginal proestrus (nucleated cells); (b) = vaginal keratinization; (c) = in the afternoon, 18 hours before traumatization. (Modified from Table 1 in ROSSI CARTONI & BIGNAMI, 1966).

		N	DAYS								
			− 8	− 7	− 6	− 5	− 4	− 3	− 2	− 1	0 = Tr, and + 1, + 2, + 3
A1	Oe	8	—	—	0.75	0.75	— (a)	0.2 (b)	0.2	0.2	0.2
	P		—	—	—	—	—	4	4	4	4
A2	Oe	7	—	—	0.75	0.75	— (a)	0.1 (b)	0.2	0.4	0.2
	P		—	—	—	—	—	4	4	4	4
A3	Oe	6	—	—	0.75	0.75	— (a)	— (b)	—	0.8 (c)	0.2
	P		—	—	—	—	—	4	4	4	4
B1	Oe	11	—	0.75	0.75	— (a)	— (b)	0.2	0.2	0.2	0.2
	P		—	—	—	—	—	4	4	4	4
B2	Oe	6	—	0.75	0.75	— (a)	— (b)	0.1	0.2	0.4	0.2
	P		—	—	—	—	—	4	4	4	4
B3	Oe	6	—	0.75	0.75	— (a)	— (b)	—	—	0.8 (c)	0.2
	P		—	—	—	—	—	4	4	4	4
C1	Oe	7	0.75	0.75	— (a)	— (b)	—	0.2	0.2	0.2	0.2
	P		—	—	—	—	4	4	4	4	4
C2	Oe	6	0.75	0.75	— (a)	— (b)	—	0.1	0.2	0.4	0.2
	P		—	—	—	—	4	4	4	4	4
C3	Oe	6	0.75	0.75	— (a)	— (b)	—	—	—	0.8 (c)	0.2
	P		—	—	—	—	4	4	4	4	4

The use of an oestrogen with shorter duration of action (non-esterified oestradiol) leads, other things being equal, to a lengthening of the real interval between the end of the follicular phase and traumatization. In fact schedule B, which leads to an optimal sensitivity in the case of the benzoate derivative, did not allow decidualization in the case of oestradiol (Fig. 2 and Table 2). Schedule A corresponded, in the case of the benzoate,

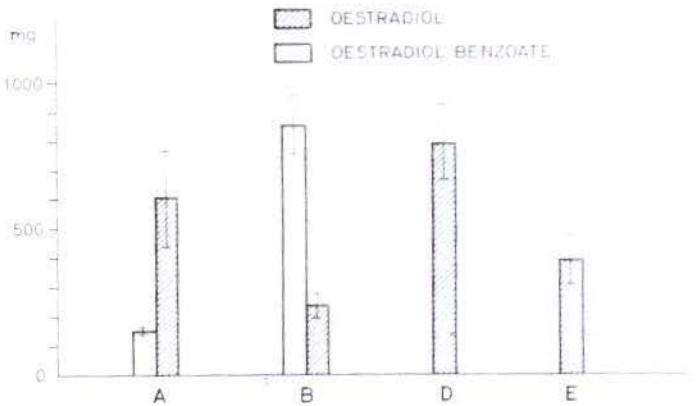

Fig. 2. — Decidualization in ovariectomized rats given daily oestrogen and progesterone treatment between the end of the follicular phase and traumatization. The use of oestrogens with different duration of action led to changes in the optimal temporal relationships. For the schedules of hormonal treatment see Table 2. The bars give the mean weight of traumatized horns (in mg) ± SE. (From De Luca, 1969).

to a real interval between follicular phase and traumatization too short for a successful induction of decidualization. The same condition allowed a fair degree of decidualization in the case of oestradiol. The latter gave still better results when the priming was prolonged (schedule D), i.e., the treatment started as in condition B and terminated as in condition A. Finally, a further prolongation of the treatment led to a decline of sensitivity even with the short-acting hormone, obviously because traumatization came again too close to the end of the follicular phase (schedule E).

The above data demonstrate that the use of a continuous oestrogen treatment during the artificial luteal phase imposes strict temporal limits.

This can well explain why previous investigators found that such treatments reduced the endometrial sensitivity below the level obtained with progesterone alone (e.g.: Rothchild & Meyer, 1942; Czyba & Dubois, 1965). Evidence in favour of an oestrogen-progestogen synergism was eagerly sought for by means of experiments in which both hormones were given before traumatization. One such typical experiment was performed

TABLE 2.

Schedules of hormonal treatment used in the experiments of Fig. 2.

Oe = Oestradiol benzoate (white bars) or oestradiol (hatched bars) (μg); P = Progesterone (mg); Tr = Traumatization day; (a) = vaginal pro-oestrus (nucleated cells); (b) = vaginal keratinization; N = 8-10 animals per group (From DE LUCA, 1969).

		DAYS							
		− 7	− 6	− 5	− 4	− 3	− 2	− 1	0 = Tr, and + 1, + 2, + 3
A	Oe	—	0.75	0.75	— (a)	0.2 (b)	0.2	0.2	0.2
	P	—	—	—	—	4	4	4	4
B	Oe	0.75	0.75	— (a)	— (b)	0.2	0.2	0.2	0.2
	P	—	—	—	—	4	4	4	4
D	Oe	0.75	0.75	0.75 (a)	— (b)	0.2	0.2	0.2	0.2
	P	—	—	—	—	4	4	4	4
E	Oe	0.75	0.75	0.75 (a)	0.75 (b)	0.2	0.2	0.2	0.2
	P	—	—	—	—	4	4	4	4

with animals ovariectomized at oestrus, treated with progesterone for 12 days, traumatized on the 8th day of the artificial luteal phase and given oestrogen for more than one day before traumatization (ROTHCHILD & MEYER, 1942). All groups showed less decidualization than the group treated with progesterone alone, except perhaps a group treated with very small doses of oestrogen for only 1.5 days before traumatization. Obviously, with the temporal relationships indicated above, only a re-introduction of oestrogen on the day before traumatization could have raised the endometrial sensitivity above the level permitted by progesterone alone.

Hypotheses about patterns of oestrogen secretion and type of neural control. — The results discussed in the previous section lead to the theoretical possibility that a peak of endometrial sensitivity on day 4 be the result of a continuous oestrogen and progesterone secretion on days 1, 2 and 3, according to the hypothesis supported by YOCHIM & DE FEO (1963). Such pattern of secretion, however, cannot account for sensitivity at any other time during the luteal phase. For example, oestrogen secretion on days 2, 3 and 4 could not lead to a high sensitivity on day 5 ; oestrogen secretion on days 8, 9 and 10 could not lead to a high sensitivity on day 11.

On the other hand, the « surge » model proposed by Shelesnyak can account for a high sensitivity at any time during a given luteal phase, excepting days 1, 2 and 3 which are too close to the previous follicular phase. The latter model has been recently accepted by DE FEO (1967) (*).

Hypotheses about the neuro-endocrine control of implantation must be discussed in the light of the various possibilities outlined above. If the « surge » model has general validity, then a relatively simple type of control can be postulated. Ovulation and mating would be followed by a relative inactivity of the gonadotrophic centers, and a predominance of prolactin secretion. A renewed secretion of FSH and/or LH would cause implantation at a time determined by the central nervous system. Lactation would retard the abrupt resumption of gonadotrophic activity taking place after a period of quiescence.

A different type of control must be postulated if one accepts the view that implantation in non-lactating animals is determined by the continuous secretion of both oestrogens and progestogens after ovulation. In this case, lactation would prevent a continuous, low-level gonadotrophic activity

(*) DE FEO (1967) still rejects the terminology proposed by Shelesnyak. His objections are based on the fact that the resumption of oestrogen secretion is probably not followed by a major fall after the period of maximal uterine sensitivity — hence the rejection of the term « surge ». As indicated in a previous section, there is good agreement that both oestrogen and progesterone contribute to the optimal growth of the decidual tissue after a successful induction of decidualization.

leading to a continuous oestrogen output. The delay would be immediately terminated upon the resumption of oestrogen secretion. If the « surge » model has general validity, the development of a high endometrial sensitivity on day 4 is the consequence of a definite chain of events - hypothalamic, pituitary, ovarian and endometrial activation in succession. In the other case the brief peak of endometrial sensitivity on day 4 should be considered the result of a relatively stable endocrine situation.

Although further experiments are needed to clarify these points, other lines of evidence have been interpreted as supporting the surge hypothesis. First, there is ample evidence that various experimental procedures (hypophysectomy ; ovariectomy ; administration of neuroleptics, or anti-LH serum, or the anti-oestrogen ethamoxytriphetol) prevent implantation in rats if performed before certain critical periods, but not afterwards (see the reviews mentioned in the introduction and, for anti-LH serum : MADHWA RAJ, SAIRAM & MOUDGAL, 1968 ; for a recent replication of the basic results in mice : BINDON, 1969). The supporters of the surge hypothesis contend that these data are in favour of the definite chain of events outlined above — hypothalamic, pituitary, ovarian and endometrial activation. The supporters of a steady output of oestrogen, however, could reply that the elimination of the last part of a continuous secretion could lead to the same results.

Other data seem to be more directly in favour of the surge hypothesis. These include the increase in ovarian volume taking place at the time at which a surge is expected (BITTON, VASSENT & PSYCHOYOS, 1965), and the trend of uterine weights during pseudopregnancy (DE FEO, 1963*a*). Both are better compatible with a sudden resumption of oestrogen secretion on day 3 than with a continuous, low-level secretion after the end of the follicular phase.

Cyclic oestrogen secretion and oestrogen surge. — The discussion in this section will be based on the assumption that it is preferable to accept the surge model in order to account for all physiologic instances of high sensitivity to deciduogenic stimuli, either on day 4 or at a later time. One additional point in the Shelesnyak hypothesis has been the object of controversy. The question is, whether or not similar mechanisms underlie the oestrogen surge of progravidity and the oestrogen secretion in the follicular cycle.

In 1929 NELSON described changes in the vaginal smears of rats during pregnancy which could be taken as evidence of an oestrogen secretion at about the normal time for implantation. Furthermore, SWEZY & EVANS (1930) found evidence of a cyclic follicular activity during pregnancy. Finally, KREHBIEL (1941) indicated that, in cases of lactational delay, the

probability of implantation was not uniform over successive days, but rose on days in which the animals would have been in oestrus in the absence of lactation and post-partum mating. Based on these data, Shelesnyak and coworkers postulated that similar mechanisms underlie oestrus cycles and the oestrogen surge of progravidity (Shelesnyak, 1960; Shelesnyak & Kraicer, 1963; Shelesnyak, Kraicer & Zeilmaker, 1963).

Various lines of evidence have been mustered to disprove this part of the Shelesnyak hypothesis. A preliminary distinction must be made before discussing this evidence. Obviously, gonadotrophic activation during pregnancy cannot be equated to that taking place in the cycling animal which shows, *inter alia*, ovulation and a high level of sexual receptivity. Therefore, we prefer to elude the general question, whether or not a regular cyclic activity takes place during pregnancy. The real question appears to be, whether or not the hypothalamo-pituitary-ovarian activation which leads to nidation is similar to that taking place at the end of dioestrus.

Consider, for example, the controversy on the data of Krehbiel (1941) mentioned above. Zeilmaker (1964) and Mantalenakis & Ketchel (1966) were unable to confirm that, in lactating animals, the probability of implantation is greater in certain days. These data simply indicate that the postponement of a given sequence of neural and endocrine events does not imply that the sequence should reappear at a time corresponding to a multiple of the original rhythm. This is not a peculiarity of the oestrogen surge of progravidity since the same happens when cyclic oestrus is delayed by experimental manipulations. For example, if a rat shows a 4-day cycle and ovulation is postponed from day x to day x + 2, successive ovulations will take place on days x + 6, x + 10, ... and not on days x + 8, x + 12, ... Therefore, once implantation has been delayed by lactation, there is no reason why it should take place at a time corresponding to a multiple of the original rhythm.

Other investigations have shown that pharmacological treatments or lesions which can block ovulation do not prevent implantation (De Feo, 1963*a*; Zeilmaker, 1963; Kordon & Psychoyos, 1966). However, block of ovulation and block of oestrogen secretion must be considered separately, and only the latter could affect the time of implantation.

Finally, it is known that implantation (or maximal sensitivity to deciduogenic stimuli) occurs at the same time in rats with a 4-day or a 5-day rhythm (see discussion in Rossi Cartoni & Bignami, 1967). It seems, however, that differences in the length of intrinsic rhythms, probably influenced by genetical and/or maternal factors and by environmental factors, are due to differences in the neurohormonal mechanisms responsible for ovulation (Everett, 1961; 1964; Alloiteau & Mayer, 1967). The interval

between ovulation and the resumption of oestrogen secretion at the end of dioestrus might be the same in 4-day or 5-day rats.

It appears clearly that the question cannot be solved on the basis of these data and that more information is needed on other points, e.g., the source of nidatory oestrogen.

Biological significance of the oestrogen-dependence of nidation. — Other things being equal, the oestrogen-dependence of implantation obviously increases the degree of control exerted by the central nervous system on nidatory processes. It appears that the Myomorph species considered here possess a series of characteristics which leads to a high level of fertility. The list includes brief oestrus cycles, brief pregnancy, large size of litter, post-partum ovulation and receptivity, and lactational delay. Short cycles, due to the absence of spontaneous pseudopregnancy, minimize the risk of non fertilization. In fact, ovulation takes place spontaneously, but the corpora lutea have a short life in the absence of mating. These endocrine phenomena appear to be correlated with the patterns of reproductive behaviour. For example, the experimental reduction of the amount of genital stimulation received by the female before the male ejaculates can prevent implantation (Wilson, Adler & Le Boeuf, 1965). Post-partum fertilization and lactational delay can be considered as a « compromise » between casting successive litters at too short intervals and postponing fertilization until the lactational anoestrus comes to an end. The studies using experimental manipulations of the delay have not yet included systematic evaluations of the consequences of its suppression. However, the observations of Bruce & East (1956) on mice indicate that a spontaneous absence of delay in animals nursing large litters can have adverse consequences on the offspring.

The above considerations suggest that a more thorough comparative study of the lactational delay and the oestrogen-dependence of implantation could throw further light on the relationships between general biological and behavioural characteristics and endocrine patterns. On the one hand, it is evident that reproductive patterns, both endocrine and behavioural, are markedly different in Myomorph and Hystricomorph rodents (see discussion in Bignami & Beach, 1968). In the guinea-pig, for example, pregnancy is much longer, implantation does not depend on the secretion of additional oestrogen after the end of the follicular phase, fertilization can take place after parturition but, apparently, there is no lactational delay of implantation. With small litters and long intervals between successive parturitions there seems to be no risk of deleterious effects on the offspring.

Even more interesting can be the comparisons between Myomorph species with similar endocrine patterns, but differing in regard to presence

or absence of post-partum fertilization and lactational delay. Hamsters, for example, do not ovulate after parturition, and can be fertilized again only at the end of the lactational anoestrus (ASDELL, 1964). Correspondingly, progesterone alone is sufficient to allow implantation (*) (PRASAD, WARD ORSINI & MEYER, 1960; WARD ORSINI & MEYER, 1962; WARD ORSINI & PSYCHOYOS, 1965; HARPER, PROSTKOFF & REEVE, 1966).

Other Myomorph species, e.g., those of the genus Peromyscus, should be investigated in order to test the hypothesis that post-partum fertilization, oestrogen-dependence of nidation and lactational delay are strictly related to each other. In fact, it is known that some Peromyscus species show post-partum fertilization and lactational delay, while others mate only at the end of the lactational anoestrus (ASDELL, 1964).

SUMMARY AND CONCLUSIONS

The data on ovoimplantation and experimental induction of decidualization show that, in certain Myomorph species, the presence of both oestrogens and progestogens is necessary to obtain a maximal endometrial sensitivity to deciduogenic stimuli. Oestrogens not only synergize with progestogens, but also lead to a state of complete refractoriness after a brief peak of high sensitivity.

The data on experimental decidualization in ovariectomized rats primed with oestrogens and treated with progesterone indicate that: 1) a high endometrial sensitivity on a day equivalent to day 4 of pregnancy or pseudopregnancy can result either from a continuous oestrogen treatment on days 1, 2 and 3, or from the reintroduction of oestrogen on the day before traumatization; 2) a high endometrial sensitivity on day 5 or later can result exclusively from the re-introduction of oestrogen on the day before traumatization. Therefore, it seems that the « oestrogen surge » hypothesis of SHELESNYAK can explain the occurrence of a high endometrial sensitivity either at the time typical for non-lactating animals, or at a later time, as observed in lactating animals. On the contrary, if the hypothesis of a continuous, low-level secretion of oestrogen after ovulation is accepted to account for a high sensitivity on day 4, then the model of SHELESNYAK must at least be accepted to account for cases of delayed implantation.

These and related data have a series of practical and theoretical implications. For example, they must direct the choice of substitutive hormonal

(*) See, however, DE FEO (1967), who points out that there is an inconsistency between this absence of oestrogen-dependence and the existence of some pace-setting mechanism. The latter is revealed by the decline of uterine sensitivity to deciduogenic stimuli after a peak reached on day 3 of pseudopregnancy.

treatments when ovariectomized animals are used to assess the mechanism o action of drugs on reproduction (see, e.g., BIGNAMI & ROSSI CARTONI, 1967). From a theoretical point of view, these data must be taken into account: *a*) when discussing possible patterns of neuro-endocrine control of nidatory processes; *b*) when evaluating the broader biological significance of the oestrogen-dependence of implantation and of lactational delay. In this regard, two types of correlational analysis could be carried out. The first considers broad sets of biological, behavioural and endocrine characteristics which appear to be correlated, e.g., when Myomorph and Hystricomorph rodents are compared (BIGNAMI and BEACH, 1968). The second considers species with closer taxonomic relationships, differing only in regard to certain characteristics, e.g. presence or absence of post-partum fertilization, lactational delay and oestrogen dependence of implantation.

The data on the comparison between oestradiol and oestradiol benzoate (Fig. 2 and Table 2) were obtained in the course of a series of experiments carried out by Dr. Eugenia De Luca and described in her unpublished dissertation (DE LUCA, 1969).

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