ones[43]. This correlates with the existence of two cell populations in PNH, a short lived, deficient one and another with longer survival. A striking difference appears in this respect between PNH cells and cells from patients affected by autoimmune hemolytic anemia. In the latter case the cells are also ACHE deficient, but the enzyme activity declines sharply with the cell age[43].

JACKSON and WHITTAKER[44] confirmed both the low acetylcholinesterase activity of PNH cells, as well as their abnormally low density previously observed by LEWIS and VINCENT[45]. The low density could not be correlated with either a change in lipid content, which was found normal, or with increased permeability, their osmotic fragility being also normal[45].

An abnormal membrane protein pattern obtained by SDS-polyacrylamide gel electrophoresis was found in a PNH patient which was severely aplastic. Other PNH cases, as well as the same patient in a hyperplastic phase, gave normal protein patterns[44].

Electron microscopy in PNH

Numerous efforts have been made to visualize the initial acquired lesion in PNH cells by electron microscopy, but these attempts have produced conflicting results with some investigators claiming to find lesions and others finding essentially normal red cell membrane ultrastructure. WEINSTEIN and WILLIAMS[46] criticize the technique used in these studies as producing drying artifacts, and studied the membrane in intact PNH cells and in the ghosts derived from them by the freeze cleaving technique. Their results failed to confirm previously reported lesions, and the authors suggest that they were either artifacts of drying, or they reflect structural differences revealed by drying.

Hemolysis associated with altered phospholipid composition of the erythrocyte

A familial nonspherocytic hemolytic anemia associated with abnormalities in membrane lipids was described by JAFFE and GOTTFRIED in 1968[47]. The patient's erythrocytes showed an absolute increase in lecithin content while the plasma lipid distribution was normal. The unusual lipid abnormality seemed to be related to the hemolysis. Later, SHOHAT et al.[48] studied the mechanism of lecithin accumulation in the erythrocytes of such patients and concluded that lecithin increases because a defect in the catabolism of actively incorporated lecithin fatty acids. This defect appears to be a block in the transfer of fatty acids from lecithin to phosphatidyl ethanolamine prior to final release from the cell. The passive exchange pathways and the active anabolic acylase in the erythrocytes of such patients were not abnormal[48].

This familial hemolytic disease with abnormal lipid composition results from an inherent membrane defect and differs from other similar states in which the primary defect is in the serum.

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Some Aspects of the Early Development and Implantation of the Mammalian Egg

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Summary. The early development and implantation of the mammalian egg is described for various species and the differing and often contradictory solutions proposed by different authors for the many problems arising from their investigations are exposed, compared and discussed.

The early development and implantation of the mammalian egg has received much attention and an immense quantity of literature has been accumulating, continuously increasing during the last decades. Nevertheless many problems are still not clear and for most of them the solutions proposed by different investigators differ widely and are often contradictory. It would be quite impossible to give an overall survey of the work dealing with the subject. The present review, which is far from complete, attempts to expose some of the problems and their solutions.

There are great differences between species, and even between strains, and although the mouse has been my main experimental animal, observations in other species will be cited when necessary.

Ovulation, fertilization and tubal passage of sperm and fertilized ova

In most animals ovulation occurs spontaneously, but in certain species it is provoked by coitus, e.g. in the rabbit, ferret, alpacca. Hamsters ovulate 8 to 9 h after the onset of oestrus. In the rabbit, after the necessary quota of gonadotrophin is released into the circulation following coitus, an interval of about 9 h is required for the maturation changes in the follicles to terminate in their rupture (EVERETT, 1964). In many species (man, mouse, guinea-pig, rabbit, dog, cat, macaque) maturation cleavage of the egg takes place also in atretic follicles (BURKL and KELLNER, 1960). In the majority of animals fertilization occurs in the oviduct, where the spermatozoa await the egg for 6 to 30 h, according to species, i.e. rabbit 6 h, ferret 24, pig, sheep, cattle 20 to 30 h (CHANG 1951). In a few species fertilization takes place in the ovary (dog, weasel), in certain insectivores even intrafollicularly, preceding ovulation (BLUNTSCHLI, 1939; STRAUSS, 1954). The tubal passage of the egg during cleavage lasts 3 to 4 days, regardless of the length of the tube. It is regulated by decreasing oestrogen and increasing progesterone output. In the mouse and rabbit, the ova can be retained in the Fallopian tube by oestrogen injections (tube-locking); on the other hand progesterone injections accelerate the passage (ASDELL, 1961). In both instances, implantation is prevented (BURDICK, WHITNEY and PINCUS, 1937; WHITNEY and BURDICK, 1938; BURDICK, 1942). The utero-tubal junction has been shown to retain the tubal fluid in the sheep, but to give passage to the spermatozoa (EDGAR and ASDELL, 1960). In the rabbit doe, rabbit sperm passed the utero-tubal orifice, but foreign sperm (rat and guinea-pig) were found dead in the vagina (CHANG and BEDFORD, 1961). In the rat also, only rat sperm are allowed to pass the utero-tubal junction, but not foreign sperm (LEONARD and PERLMAN, 1949).

Capacitation of spermatozoa

Spermatozoa undergo certain physiological changes in the female genital tract before they are capable of penetrating the zona pellucida and fertilizing the ovum. Phagocytosis of redundant sperm, development of the fertilizing power of selected ones and biochemical reactions in the uterine lumen constitute the phenomena observed in the rat and rabbit, termed capacitation by AUSTIN, which has been known since 1951. It has been found in all mammals so far studied (rat, mouse, hamster, sheep, ferret), but is still obscure. 2 to 10 h are required in different species for capacitation to be accomplished. After that period, the spermatozoa retain their fertilizing capacity for up to 48 h, in the mouse 6, rat 14, guinea-pig 22, rabbit and farm animals 24 to 48 h. Exceptions are the horse with 6 days and the bat with 156 days (CHANG, 1951, 1955, 1958, 1965; ADAMS and CHANG, 1962). Whereas the fertilizing capacity is said to be impaired by rise in temperature (short exposure of testicles to a higher temperature cause severe damage to spermatogenesis (CHANG, 1955), bovine sperm could be stored for 20 weeks at —79 °C and then used for artificial insemination. In the bat, where spermatozoa keep their fertilizing capacity for several months in the genital tract of the female, it is suggested that the low temperature of the hibernating female keeps them alive (OTTOW, 1953). CHANG (1955, 1959) has reviewed the phenomenon of the prodigality of spermatozoa (5000 millions are ejaculated at mating by the bull, 500 millions by man, 200 millions by the rabbit) and the explanation given by different workers for the need of these great numbers, e.g.: dispersion of the cumulus cells surrounding the egg by the spermatic enzyme hyaluronidase, the vast area of the internal surface of the female tract, the speed of disintegration in the uterus, the heterogeneous quality of the spermatozoa constituing a sperm population. All these explanations can be refuted. CHANG's review of the different problems associated with the fertilizability of the male and female germ cells leads him to the conclusion that our understanding of mammalian fertilization is very elementary.

The spacing of ova

The spacing of ova, their regular distribution along the length of the uterine horns of polytocous animals, is another problem not fully understood. As soon as the fertilized eggs have entered the uterus, they are more or less regularly dispersed along the horns where they stay until nidation occurs. While some authors do not find an explanation for the phenomenon (WIDAKOWITCH, 1911; PARKER, 1931), others propose different solutions: An uterine segment 'saturated' by an implanting embryo repells the following ones and lets them pass (OTTOW, 1953), each implanting ovum by its relation with the mucosa renders the immediate neighbourhood refractory to any other embryo (MOSSMAN, 1937), simple stirring brought about by uterine movement (MCLAREN and MITCHIE, 1959, mouse), after being scattered randomly by uterine muscle, the expanding blastocysts cause the uterus to space them at equal distancies (BÖVING, 1950, rabbit), the spacing mechanism appears to be a progesterone-regulated stimulus-effector system (BÖVING, 1956, rabbit). The last two differing explanations by the same author and concerning the same animal demonstrate the difficulty of understanding the problem.

In my experience with the mouse (BLOCH, 1966), I obtained the same results as KREHBIEL (1941) and FRAZER (1955) for the rat and MCLAREN and MITCHIE

(1959) for the mouse, namely that the regular spacing is only apparent. The eggs are distributed down the length of the horn, probably by muscular contractions of the uterus. Each blastocyst implants at the next free site. The increasing size of the growing embryos occupying all the available space, especially when they are numerous, brings about the aspect of equally spaced swellings. But irregularly distributed implantation sites are also found (Figure 1).

Preimplantation stages of the uterus and uterus-blastocyst interaction

Considering metabolic and hormonal needs for nidation, which differ between species, the question of preformed implantation sites in the uterus arises. In the cow, there are definite implantation sites (caruncles); in the horse endometrial cups, in the human female the fundus uteri seems to be the preferred site. From the arrangement of the blood vessels in the centetidae, it can be concluded that the endometrium is divided into various lobules where the ovum attaches itself (STRAUSS, 1944). In the hamster, certain districts of the antimesometrial endometrium containing more ribonucleic protein are the implantation sites (STRAUSS, 1956). In the African shrew *Elephantulus*, where 120 ova are shed during ovulation, only one implants near the cervix (VAN DER HORST and GILLMAN, 1941). In the rat, the decidual response of the mucous membrane is different in the mesometrial and antimesometrial region where implantation occurs; it appears first antimesometrially where the cells undergo hypertrophy and hyperplasia, and accumulate intercellular lipids and glycogen (KREHBIEL, 1937). The orientation of the implanting ovum in the uterus presumes a certain preformation of the endometrium, namely antimesometrially: certain rodents, bats and insectivores, mesometrially: some megachiropteres, laterally: centetes and hemicentetes (BLUNTSCHLI, 1939; MAYER, 1953).

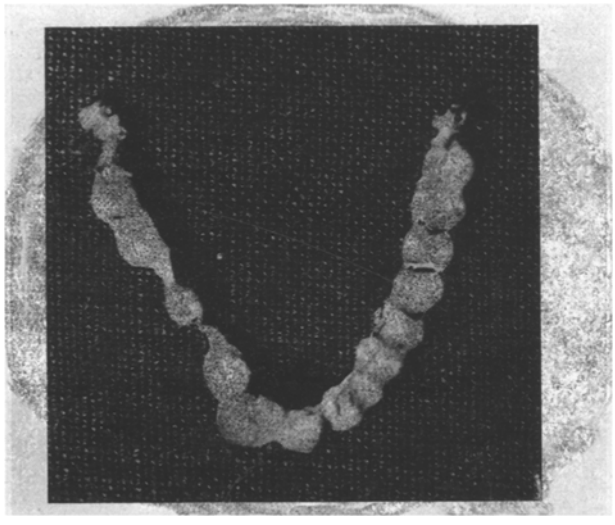


Fig. 1. Uterus of a mouse, 10th day of pregnancy. In the right horn 9 implantation sites are regularly spaced. In the left horn 5 swellings are irregularly distributed.

The uterine-blastocyst interrelationship termed by MOSSMAN (1937) 'a set of interactions by the embryo and the maternal organism' has been the subject of numerous investigations, but nevertheless still remains obscure due to conflicting experimental results. Some authors attribute the active role to the uterus, others to the blastocyst, still others to the interaction of the 2 partners. Here some examples: 1. Active role of the uterus: The factor limiting the number of blastocysts which can develop in any individual depends on the amount of a specific growth substance present in the uterus (HAMMOND, 1959).

In the mouse an 'uterine factor' enables the ova to accomplish implantation and further development (KIRBY, 1962). Only blastocysts extracted from the uterus will, when transplanted to an extrauterine site, develop into embryos. A blastocyst, in order to realize totipotency, must be subjected to the environment of a hormonally receptive uterus (KIRBY, 1965). The invasion of the blastocyst has been described as unilaterally active. The author considers the blastocyst only as an inductor of a process which is specific for the endometrium (human female) (SCHMIDT-MATHISSEN, 1968). Possibly the stimulus which induces a local increase in capillary permeability also induces secretion of a zona lysin by the uterus (MCLAREN, 1967).

2. Active role of the blastocyst: It is suggested that the contact with the cell membrane of the trophoblast influences differentiation of the uterine epithelium of the mouse (POLLARD and FINN, 1974). The blastocyst supplies the oestrogens necessary for implantation (rat) (KEHL and CHAMBON, 1949). The decidual reaction begins after the destruction of the epithelial surface by the trophoblast (hamster) (SCHENK, 1957). The 4-day pseudopregnant mouse uterus has the competence to react to the blastocyst which stimulates the epithelium already before being in contact (FINN and HINCHLIFFE, 1965). As the first step towards ovo-implantation in the mouse, the blastocyst induces the formation of a spherical crypt in the endometrium (BLOCH, 1966, Figure 2).

3. Uterine-blastocyst mutual interaction: The present studies cannot dissociate the egg from the influence of the uterus. The blastocysts are activated by the same hormonal stimulus that prepares the uterus to receive them (mouse) (WEITLAUFF and GREENWALD, 1968). Active blastocysts of rats were transferred into dormant uteri and dormant blastocysts transferred into sensitive uteri. In both types of transfers, the blastocysts developed into normal fetuses (DICKMAN and DE FEO, 1967).

Probably both partners collaborate and each of them contributes to the interaction. The active role

of the blastocyst is ascertained by the possibility of ectopic implantation (duodenum, anterior chamber of the eye, testes, seminal vesicle, liver, also in castrates). But, on the other hand, the activity of the uterus is shown by the possibility of obtaining decidualization by foreign bodies.

Decidualization

Decidualization of the endometrium, the first step of implantation, can be experimentally induced by different means: electrical stimulation, threads inserted into the uterine wall, injury of the mucosa (scratching, crushing), injected air or oil, chemical and other traumatizations. But the process of decidualization in the non-pregnant female is not quite identical with the decidualization which accompanies implantation. In the ovariectomized guinea-pig, in which nidation may occur without exogenous hormones (Deanesly, 1960), deciduoma formation cannot be realized without progesterone (Deanesly, 1961). Here again species differences are reported. In the rat, deciduomata can be stimulated in the lactating female while implantation does not occur (Krehbiel, 1941). Deciduomata form in rats spayed on the 4th day p.c. receiving progesterone, but for ovonidation additional oestrogen is required (Mayer, Thévenot-Duluc and Meunier, 1959). In the spayed rabbit deciduoma production, though not ovo-implantation, requires oestrogen as well as progesterone (Kehl and Chambon, 1950). Deciduomata may even inhibit implantation (Mayer and Klein, 1946; Mayer, 1960; Nicholson, 1968; Meyer and Cochrane, 1962).

The zona pellucida

The blastocyst is surrounded by a mucoprotein coat whose formation and fate varies among different species and according to different authors. In the rat, it is described as a non-cellular envelope keeping the blastomeres together and preventing their fusion during the passage through the uterus which would lead to production of chimaeras (Mayer, 1969). However, during most of the period of delayed nidation in suckling mice, the blastocysts remain in the uterus free of the zona (McLaren, 1967). The shedding of the zona is a prerequisite for nidation, but not in itself a sufficient condition (Strauss, 1959), and delayed implantation is not related to the persistence of the zona (McLaren, 1967). The mechanism of shedding of the zona is ascribed to digestion by uterine fluids in the rabbit (Pincus, 1936), to expansion by pressure of the inner liquid of the blastocyst in the guinea-pig (Blandau, 1949), it is dependent on oestrogen in the rat (Mayer, 1969; Psychoyos, 1966), on progesterone in the hamster (Orsini, 1963) and on lysin, a proteolytic enzyme secreted by the uterus in the mouse (Bowman and McLaren, 1970). The zona disappears by hatching of the blastocyst in the rat (Mayer, 1969), by active hatching, but with an additional hormonally determined uterine factor in the mouse (Orsini and McLaren, 1967), by a lytic factor emanating from the oestrogen-sensitized uterus and the expansive activity of the blastocyst itself in the mouse (McLaren, 1970). Different other influences causing the shedding of the zona are suggested (Hafez, 1963).

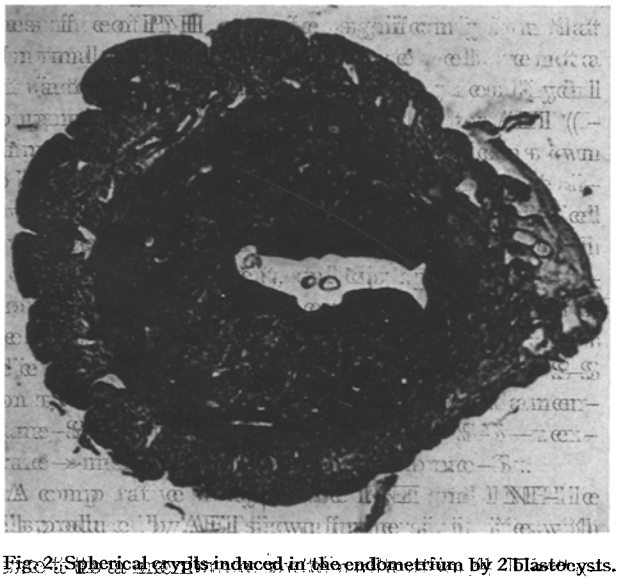


Fig. 2. Spherical crypts induced in the endometrium by 2 blastocysts.

Ovo-implantation (nidation)

Implantation which terminates progestation and initiates gestation is an important step in the course of pregnancy. There are many differences between the preimplantation stage and the gestation period. Hormone requirements are not identical before and after nidation. The pregnancy block brought about in newly mated mice by the odour of strange males (Bruce effect) as well as various traumatizations such as auditory stimuli, crowding, immobilization, handling, are effective only during preimplantation by inhibiting nidation (Bloch and Rippmann, 1968). During preimplantation, fertilized ova can be flushed out of the Fallopian tube, stored in vitro and transplanted into pseudopregnant recipients. Chang and Marden (1954) shipped 2-cell and 4-cell rabbit ova fertilized in the United States by rail and air to England where they were transplanted into pseudopregnant does and developed to full-term rabbits.

Implantation is the beginning of the intimate connection of the developing embryo with the maternal organism. It has been proposed to consider nidation as the beginning of pregnancy, but the female carrying blastocysts in her genital tract must be considered pregnant already in the preimplantation period.

The importance of understanding the process of nidation is suggested by the fact that about half of the fertilized eggs fail to survive it, the time of implanta-

tion representing the greatest pregnancy wastage and the highest death rate of the human life span (BÖVING, 1959). Implantation in nearly all mammals is dependent on the ovarian hormones, viz. on progesterone and a small amount of oestrogen. In most species nidation in ovariectomized females is not brought about without injection of both hormones. In the hamster maintenance of the blastocyst and nidation could be obtained with progesterone alone (PRASAD, ORSINI and MEYER, 1960). In the guinea-pig where blastocysts implant in the ovariectomized female, it is suggested that the amount of ovarian hormones required for nidation is exceptionally low or that they are supplied by other sources, possibly the adrenals (DEANESLY, 1960). The French school (COURRIER and his co-workers) emphasize the importance of the balance of the two ovarian hormones. Some investigaters (SHELESNYAK and his group) postulate for the rat an 'oestrogen surge' occurring in the afternoon of the 4th day p.c., whereas others, including the present writer, believe that the oestrogen secreted at ovulation, extends its effect to the time of implantation (BLOCH, 1966, 1968), perhaps sustained by continuous secretion of low levels of the hormone (FINN and MARTIN, 1969). Arguments for both theories are presented. The finding that the decidual response to a single injection of oestradiol was not so great as that to multiple small doses (FINN and MARTIN, 1969), that an injection of oestrogen given the first day p.c. was sufficient to bring about nidation on time (BLOCH, 1968) in mated suckling mice, as did daily small doses during the preimplantation period (BLOCH, 1966), that implantation could be brought about with progesterone alone after ovariectomy on the 1st to 3rd day (BLOCH, 1959) are all in favour of the theory that the oestrogen of the post-partum ovulation is sufficient for inducing nidation. On the other hand, SHELESNYAK and his co-workers have produced an impressive body of literature on the 'oestrogen surge', examining morphological, physiological and biochemical parameters (SHELESNYAK and KRAICER, 1963). So it seems difficult to decide which one of the two theories can be regarded as proven.

Delayed nidation

a) Naturally or normally occurring delay ('nidation différée' of the French authors). In the majority of mammals, nidation occurs in the beginning of the gestation period when the fertilized ova have reached their site in the uterus; but in some animals it takes place much later, in the bovines at 40 to 50 days, in the horse after 15 weeks. In some species the blastocysts remain 'dormant' in the uterine cavity without implanting for a surprisingly long time. This delay was discovered by the British anatomist JOHN HUNTER (1728–1793) in the roe-deer. Delays of up to 10 months have been described in the bear, marten, badger, mink, weasel, fur seal and armadillo. Some investigators ascribe the delay to a deficiency of progesterone secretion or to a change in the balance of the ovarian hormones (HANSSON, 1947), but the delay could not be shortened in the mink either by administration of progesterone with or without oestrogen, or by administration of oestrogen alone or in combination with progesterone (COCHRANE and SHACKELFORD, 1963). This problem still requires further investigation.

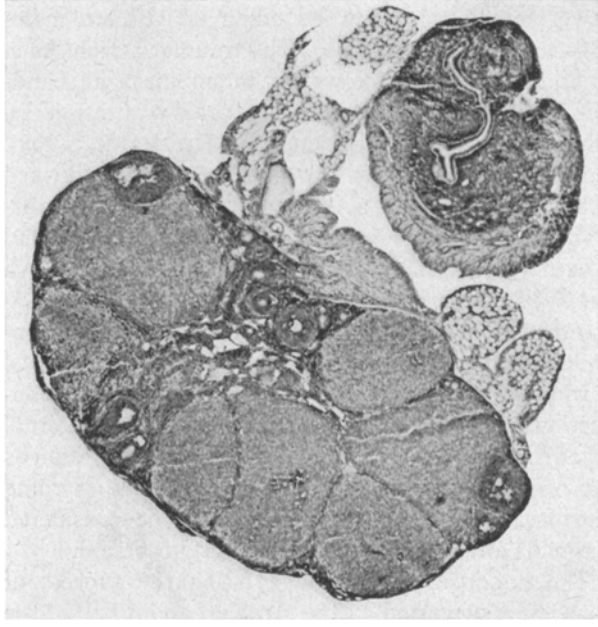


Fig. 3. Right ovary of a suckling mouse, 8th day of pregnancy.

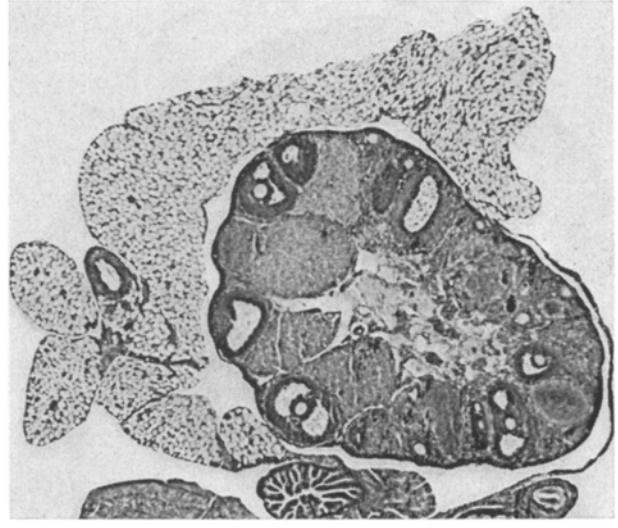


Fig. 4. Left ovary of the same female after cessation of lactation.

b) Induced delay of implantation ('nidation retardée' of the French authors). Another type of delayed implantation has been observed in the rat and mouse and a few related species when the female, impregnated at the postpartum oestrus, is suckling a litter of a certain size or rather of a certain weight (BLOCH, 1948). Also this phenomenon, first described by the French zoologist LATASTE (1886) and often experimentally investigated, is ascribed by some authors to a deficiency of progesterone, by others to a reduced secretion of oestrogen. In my experience, resulting from numerous experiments in mice and rats, the delay can be shortened and nidation induced by injections of oestrogen (BLOCH, 1958, 1959, 1966, 1968), so I conclude that this hormone is missing during the lactational delay. On-time nidation was also induced in my experiments in suckling mice by the presence of strange males which, I suggest, provokes an oestrogen secretion (BLOCH, 1971, 1973). The histological picture of the ovaries of suckling mice, showing only corpora lutea and very young follicles but a full set of active follicles after cessation of lactation, demonstrates that during lactation no oestrogens are secreted (BLOCH, 1958, Figures 3 and 4). Experiments in which I administered small doses of oestrogen to suckling mice induced a higher mortality rate in the suckled young and an oestrous aspect of their uteri (BLOCH, 1954). These effects, also found by other investigators (DE JONG, 1933; HAIN, 1935; MEITES and TURNER, 1942; WEICHERT and KERRIGAN, 1942), make it comprehensible that no oestrogens are secreted by lactating mothers, thus causing nidation to be delayed, though they do not explain how the change in hormone secretion is brought about. Lactational delay of implantation was also observed in marsupials (AMOROSO, 1959) which have dormant blastocysts in the uterus while carrying sucking young in the pouch. In seals, according to some authors (CANIVENE, 1960) lactation does not interfere with oestrus and nidation, while LEBLOND (1950) relates that the first gestation of a female seal lasts 9 months, the following ones 12 months, the prolongation probably due to lactation. This might be the rule in free-living rodents and insectivores which probably mate at every post-partum oestrus. In free-living common shrews, a species in which it is possible to distinguish between primigravidae and parous females, BRAMBELL (1937) found among the former category 24%, among the latter ones 65% carrying unimplanted ova or blastocysts in their genital tract. In the rabbit, an animal without an oestrous cycle, there is no lactational delay, but the blastocysts die when the newly mated mother is suckling a certain number of pups (HAMMOND, 1925). In the rat, delay of nidation has also been brought about by undercooling of the female and by tranquillizers which postpone the discharge of oestrogen and activate the corpus luteum.

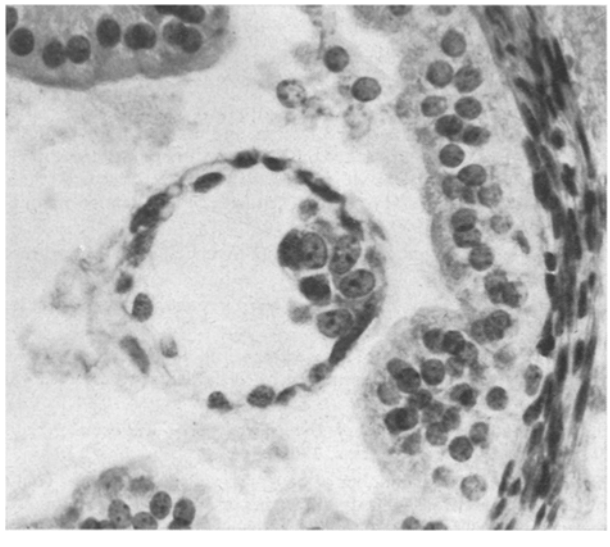
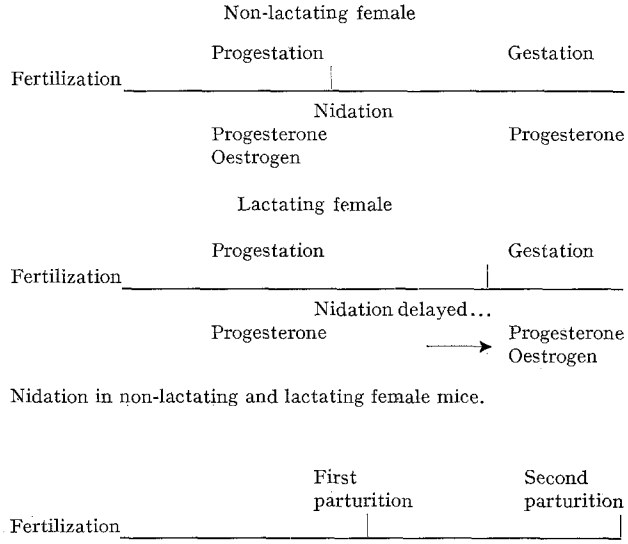
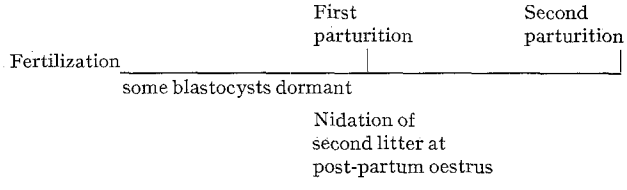


Fig. 5. Blastocyst in the oviduct, 9th day of pregnancy.



Nidation in non-lactating and lactating female mice.



Nidation of dormant blastocysts at the post-partum oestrus.

It seems that the naturally occurring delay of nidation is not identical with the lactational delay. By histochemical analysis of the endometrium of the armadillo and mink during the natural delay, and of the rat during the lactational delay, it was shown that there are considerable variations in the endometrial tissue between the three species (ENDERS, 1961).

The question arises what brings about nidation at the end of the lactational delay. It can be assumed that during lactation the release of progesterone by the corpora lutea lactationis inhibits the action of oestrogen and that, when milk secretion is declining, the oestrogen emanating from the general ovarian tissue and probably other sources (CORNER, 1965) permits implantation.

Superfetation

Superfetation, the impregnation of a female already pregnant, may occur spontaneously, but rarely, in the mink (HANSSON, 1947) and often in the hare (BLOCH, HEDRICK, LLOYD, MÜLLER and STRAUSS, 1967), but has not been proven in other species. The experimental induction of superfetation is difficult to obtain, but has been achieved in the mouse by injection of gonadotrophins (EDWARDS and FOWLER, 1958) and in the rat by local injection of progesterone into the uterine wall (CANIVENC, DROUVILLÉ and MAYER, 1953). But many records of occurrence of superfetation can be explained by belated nidation of blastocysts. The bearing of a second litter without mating after the first parturition which occurred, e.g. in 2% of 473 pregnancies in rats and mice (BURROWS, 1941), and has been described for other animals and also for women may often be mistaken for superfetation. In mice I found blastocysts in the oviduct of females carrying normally developed embryos in the other uterine horn, the difference in development being several days (BLOCH, 1952, Figures 5 and 6). This phenomenon was provoked experimentally by ligature of the utero-tubal junction, it may also occur spontaneously if blastocysts are tube-locked by a mechanical obstruction or a hormonal deficiency and then implant at the post-partum oestrus. The same phenomenon might account for observations described as telegony.

Parthenogenetic activation of eggs

Eggs can be artificially activated in vitro or in vivo by different means: heating, cooling, hypertonic or hypotonic solutions. Some of them can accomplish the first cleaving steps up to the blastocyst stage. In rare cases complete development may be achieved.

PINCUS (1939) obtained 2 female rabbits from eggs artificially activated and transplanted into pseudopregnant recipients. PINCUS and SHAPIRO (1940) parthenogenetically activated rabbit ova by cooling the Fallopian tube of a superovulated non-mated doe by water circulating in a metal jacket surrounding the tube. One of some 220 ovulated ova developed into a living parthenogenetic female rabbit.

Surprisingly these remarkable experiments have not been repeated by the same or other investigators. Though they were conducted very carefully there remains the possibility that the parthenogenetic young might have developed from ova fertilized by a previous mating of the mother which had remained in a dormant condition in her genital tract. The experiments ought to be repeated with virgin does in order to exclude this possibility.

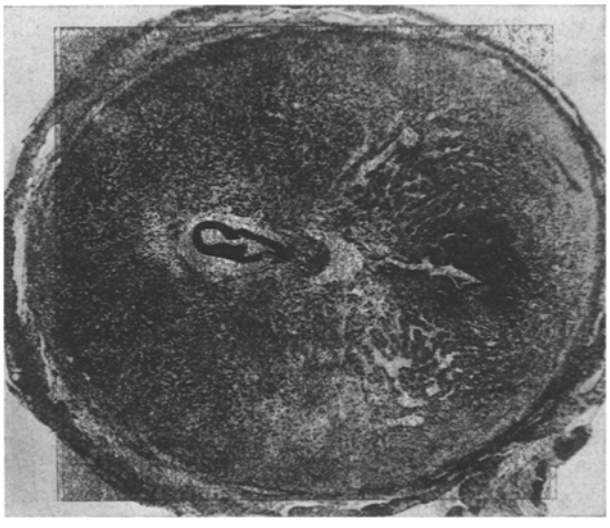


Fig. 6. Normally developed embryo in the uterine horn of the same female as Figure 5.

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Because of the great number of papers consulted, references could not be cited in extenso. Only a few papers or books containing a great list of references are named. Readers interested in certain subjects may ask the author for references or reprints if available.