THE MITOTIC ACTIVITY OF THE UTERINE EPITHELIUM IN MICE AT DIFFERENT PERIODS OF PREGNANCY

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(Received December 31, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

The present investigation is the continuation of previous work [2] in which it was shown that changes in the mitotic activity of the epithelium of the mucous membrane of the uterus in mice in the course of the sexual cycle may be expressed by a biphasic curve. Under these circumstances the first rise in mitotic activity is observed in proestrus and the second immediately after the end of estrus, i.e., in the first days after ovulation and the formation of the corpora lutea. The first increase in the number of mitoses during proestrus may be explained by the increased concentration of estrogens in the blood, which is in accordance with data in the literature [1]. The problem of the participation of the corpus luteum hormone — progesterone — in the regulation of the mitotic activity of the test tissue remains uncertain, for the view exists that the corpora lutea in rats and mice become functionally active only after copulation or certain other influences [5].

We decided to investigate the mitotic activity of the epithelium of the mucous membrane of the uterus in the earliest periods of pregnancy, when natural activation of the corpora lutea takes place. We were also interested in the question of parallelism in the changes in the mitotic activity of the uterine epithelium and the morphological reconstruction of the mucous membrane in connection with the formation of decidual tissue and resorption of the epithelium. We have previously shown [3] that transplantation of Ehrlich's adenocarcinoma in mice has a depressing action on mitoses in the uterine epithelium. In our research we therefore describe some results from the study of the mitotic activity in the epithelium of the uterine mucosa in pregnant mice after transplantation of a tumor.

Comparatively little work has been done on the study of the mitotic regime of the body during pregnancy. Lane [9] carried out an extensive investigation of the mitotic activity of the follicular apparatus of the ovaries in pregnant rats, and found changes in the number of mitoses in the cells of the zona granulosa; he explained these changes by variations in the quantity of gonadotropins produced by the anterior lobe of the hypophysis and the placenta. Ladman [8] showed an increase in the number of cell divisions in the anterior lobe of the hypophysis in mice, starting on the 9th-10th day of pregnancy which, in his opinion, was due to an increase in the quantity of estrogens circulating in the blood. S. S. Laguchev [4] observed a rise of the mitotic activity of the epithelium of the mammary glands in mice on the 4th, and especially on the 8th, 15th and 18th days of pregnancy. According to I. A. Alov's findings [1], the increased blood concentration of estrogens in mice during pregnancy has a depressing action on cell division in several organs not directly connected with the reproductive system (the epithelium of the cornea, tongue and intestine).

The papers cited are of undoubted interest and, on the whole, confirm the view of the important role of the estrogens in the regulation of cell division. At the same time, the problem of the participation of the corpus luteum hormone in this process during pregnancy remanins untouched in the literature.

EXPERIMENTAL METHOD

Investigations were carried out on white mice weighing 20-25 g. Altogether 200 female mice were used in the experiments; in some of these animals an Ehrlich's adenocarcinoma was transplanted subcutaneously (0.2 ml of ascitic fluid). A preliminary investigation of the sexual cycle of the female mice was made, using the method of vaginal smears, and in the stage of early estrus they were paired with male mice. A positive vaginal test was used as evidence of copulation. The inseminated females were sacrificed on the 2nd, 3rd, 5th and 8th days of pregnancy, always between 11:00 and 13:00 hours. Mice with a transplanted tumor were sacrificed on the 3rd and 8th days of pregnancy (on the 12th-14th day of growth of the tumor). Vaginal smears were taken daily throughout the experiment.

The uterus, oviduct and ovaries of the pregnant mice were fixed in Zenker's fluid. Transverse serial section (7 μ) through the upper half of the right cornu of the uterus were stained with Carazzi's hematoxylin; the number of mitoses in the epithelium of the mucous membrane was counted in every fourth section, as described in our previous paper [2]. The mitotic coefficient was determined as the number of mitoses per unit area of epithelium.

The periods of pregnancy were identified by the stage of development of the embryos, and for this purpose, in a number a cases serial sections were cut of the oviduct and uterus; the stages of development were identified from the table given in the paper by Sobotta [11-13] and Burckhard [6].

EXPERIMENTAL RESULTS

Investigation of the vaginal smears showed that even on the 3rd day after copulation all the experimental mice had passed into a state of diestrus. On the 2nd day after copulation the diameter of the uterine cornu was considerable smaller than in estrus. The lumen of the cornu was relatively large; its contours were irregular, sometimes with large inlets. The epithelial layer was mainly ill-defined from the underlying tissue. The epithelial cells were cubical and cylindrical and their nuclei usually polymorphic and irregularly stained. Signs of pyknosis and rhexis of the nuclei were frequent. Sloughing of individual cells and, less often, of portions of the epithelial layer into the cavity of the uterus were observed. The stroma was compact and its cells mainly round; the uterine glands were poorly developed. Embryos in the two-blastomere stage could be found in the oviducts at this time.

The mitotic coefficient in the epithelium of the uterine mucosa at this period of pregnancy was 0.206 (Table 1). It must be pointed out that the mitotic coefficient in normal mice during estrus, i.e., at the moment of copulation, is close to zero [2].

TABLE 1

Mitotic Coefficient in the Epithelium of the Uterine Mucosa in Mice at Different Periods of Pregnancy

Days			
2nd	3rd	5th	8th
0,206 (15)	0,282 (9)	0,005 (8)	0,000 (9)

Note to Tables 1 and 2. The number of animals is given in brackets.

On the 3rd day after copulation the surface of the epithelial layer became obviously covered with tubercles. The cells of the epithelial layer became high-prismatic; their nuclei were elongated and evenly stained. The stroma was still more compact, especially close to the lumen. The glands underwent considerable development. These changes in the endometrium were specific for the active lutein phase of the ovary and are known as progestin changes [5]. In the oviducts were found embryos at the 8-16 blastomere stage, indicating a pregnancy of 48-60 hours. The mitotic coefficient in the epithelium of the uterine mucosa was 0.282, i.e., it had altered only little in the course of the previous 24 hours.

On the 5th day after copulation the cavity of the uterine cornu was placed eccentrically, constricted and elongated in the direction of the mesometrium. The epithelial layer was quite clearly demarcated from the underlying tissue. Its cells were cubical and cylindrical, with round or slightly oval, evenly stained nuclei, displaced toward the basal part of the cell. Blastocysts lay freely in the cavity of the uterine cornu, situated in the antimesometrial or, less often, in the central portion of the lumen. From their external appearance they could be identified as belonging to the beginning or middle of the 5th day of pregnancy. The stroma near the

lumen was very compact and consisted of round cells; at the periphery they were more loosely arranged. There were large numbers of glands. The vascular network was well developed. The mitotic coefficient in the epithelium of the uterine mucosa on the 5th day of pregnancy had fallen to 0.005 .

At the beginning of the 8th day after copulation embryos were found in the stage of a rudimentary allantois, surrounded by giant cells of decidual tissue. The uterine cornua were greatly thickened and the sites of implantation were clearly visible to the naked eye. The uterine cavity was displaced by the proliferating decidual tissue toward the mesometrium. This was shown in transverse sections in the form of a very small, narrow lumen, lined with a flat epithelium. Cell division in the epithelium was absent (see Table 1).

Such were the changes in the mitotic activity of the epithelium of the uterine mucosa in the early periods of pregnancy, up to its involution.

In the mice with 12-to 14-day-old tumors(Table 2), on the 3rd day of pregnancy the mitotic coefficient in the uterine epithelium was more than halved (P = 0.05).

TABLE 2

Mitotic Coefficient in the Epithelium of the Uterine Mucosa in Pregnant Mice with Transplanted Tumors

Description of group	Mitotic coefficient in early periods of pregnancy	
	3rd day	8th day
Healthy mice	0,282 (9)	0,000 (9)
Mice with tumors	0,125 (9)	0,000 (7)

This result agrees with our previous findings: after transplantation of a tumor into nonpregnant mice, a significant fall in the mitotic activity in the epithelium of the uterine mucosa was found on the 20th day of growth of the tumor, and on the 10th day merely a tendency for this to fall was observed [3] .

It is interesting that, whereas on the 3rd day of pregnancy we observed a definite reaction on the part of the uterus to growth of the tumor (a fall in the number of cell divisions in the epithelium), cleavage of the ova in the oviducts of these mice took place at the same times as in normal animals, and no visible changes were observed in the structure of the blastomeres.

On the 8th day of pregnancy there was complete absence of mitoses in the epithelium of the uterine mucosa of the mice with 12-to14-day-old tumors,as in normal animals (see Table 2) .

In normal pregnant mice we observed a slight rise in the mitotic activity of the epithelium of the uterine mucosa on the 2nd and 3rd days of pregnancy by comparison with the usual mitotic activity during estrus [2], and by the 5th day this had fallen to a very low value. Soon after this period involution of the cavity of the uterus began, with gradual resorption of the epithelium.

The findings obtained show that the changes in mitotic activity corresponded to the morphological reconstruction of the epithelium of the uterine mucosa in the early periods of pregnancy. Confirmation was thus obtained for our previous hypothesis that the mitotic regime may reflect the functional state of an organ.

On the 2nd and 3rd day of pregnancy the mitotic coefficient in the uterine epithelium was 0.206 and 0.282 respectively; these values were very close to the value of the mitotic coefficient (0.23) in nonpregnant mice during the change from estrus to metestrus,i.e., during the same periods after ovulation [2]. This suggests that in our experiments the activation of the corpora lutea after copulation,i.e., the beginning of the appearance of progesterone in the blood stream, did not affect the mitotic activity of the uterine epithelium. This does not mean, however, that progesterone in genral plays no part in the regulation of cell division.

Lloyd [10], for instance, in experiments on castrated rabbits, found an increase in the number of mitoses in the uterine epithelium by almost 50 times after administration of physiological doses of progesterone for 3 days. Progesterone, therefore, is capable of a stimulating action on mitosis in the uterine epithelium. It is also known that the "free" progesterone content of the plasma in mice increases considerably on the 7th-8th day of pregnancy [7]. It seems to us, however, that on account of implantation of the embryo and reconstruction of the endometrium, the epithelium is then in no condition to response, as a structure undergoing resorption, to the action of progesterone by an increase in the number of cell divisions. This suggest that the participation of the sex hormones in the regulation of mitotic cell division in the body is much more complex than might have thought on the basis of the existing data. In order to elucidate further the character of this influence, experiments must be performed in which estrogens and progesterone are given to castrated animals. The results of such experiments will be the subject of our next communication.

SUMMARY

In comparison with the usual mitotic activity during estrus, the mitotic activity of the epithelium uterine mucous membrane rises on the 2nd and 3rd days, decreases considerably on the 5th and ceases entirely on the 8th day of pregnancy. These changes of mitotic activity correspond to the morphological endometrial readjusment associated with the formation of decidual tissue and epithelial resorption. In mice with inoculated Ehrlich's adenocarcinoma the mitotic coefficient decreased in the epithelium of the uterine mucous membrane on the third day of pregnancy. No disturbances of the cleavage process nor changes in the blastomere structure were noted.

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* Original Russian pagination. See C.B. Translation.