CHANGES IN THE GENITAL TRACT DURING ESTRUS

COMMUNICATION III. THE EFFECT OF INSEMINATION

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A considerable amount of indirect evidence has been published to the effect that insemination is a powerful stimulus to the female sexual system. Sperm has been shown to have an effect on isolated organs and tissue [7, 9], biologically active substances such as the enzymes hyaluronidase and mucinase [5] have been found in it, and the effect of insemination on female sexual function has been demonstrated [4, 8]. It must, however, be pointed out that there is no direct evidence of the response of the female genital tract to insemination. The difficulty is that in the uterus and vagina during estrus, i. e., at the time when insemination normally occurs, destructive changes occur in the mucous membrane and these alternate with periods of proliferative repair in the postestrus periods. For mice and rats [6, 10, 3], these histological changes have been described broadly as follows. During the proliferative phase, there is an increased rate of cell division of the epithelium of the genital tract. The epithelium of the vagina becomes reduced from two layers to one, and its outer part becomes cuticularized. There is a hypertrophy of the mucous membrane of the uterus, and the epithelial cells become regularly arranged side by side. The mucous membrane secretes actively, the stroma is loose, and the uterine glands have a large lumen. During the destructive phase there is a mass infiltration of leukocytes into the wall of the tract, and many enter the lumen. The superficial layer of the vaginal epithelium comes away. The uterine epithelium becomes uneven, the outlines of the cells and the basement membrane become less distinct, vacuoles, granules, etc. appear in the lumen, and villus-like outgrowths are formed from which cells break off, often together with the leukocytes which have penetrated into them. Until now,the biological significance of these changes has not been sufficiently well understood. We suppose that they constitute the morphological basis of the processes occurring at the same time in the lumen of the genital tract. The occurrence of the proliferative phase facilitates the reception of the ejaculate, the activation of the sperm [1], and its movement along the tract. After fertilization, an active destruction of the contents of the tract occurs, and the vaginal plug and ejaculate are voided to the outside [2]. These processes are brought about by destructive infiltration of the vaginal and uterine walls. This destruction is a preparation for the reformation of the mucous membrane and its subsequent proliferation. The latter comes to constitute a new proliferative phase in cases where the cycle is not completed by fertilization; when this does occur, the proliferative changes represent an adaption to implantation. Thus, all these changes in the wall of the genital tract, which we have described by the term "estrus complex", constitute an essential part of the sexual cycle of the female and play an important part in the reproductive process. On account of these changes, and in particular on account of the destructive infiltration, it is not possible to elicit reactive changes in the uterus and vagina in response to insemination, or to find the nature of any such changes.

In order to overcome this difficulty, we used females which were serviced outside estrus and after ovariectomy. The mucous membrane of the genital tract of these animals was in the resting condition, and reactive changes in it could be easily demonstrated. Five experiments were carried out. The animals were killed 7-10 hours after being serviced; the ovaries, vagina, and uterus were fixed in Bouin's fluid or in Zenker-formol, and embedded in

paraffin. Cross sections and longitudinal sections of the organs were stained in hemalumeosin. The sexual organs of 26 virgin females were taken as controls, of which 20 were normal (in different stages of the sexual cycle), and 6 were ovariectomized.

In the 1st experiment, 8 females were serviced at an early stage of proestrus. In one case, histological examination showed a massive infiltration of the uterine stroma and epithelium with polymorphonuclear leukocytes, many of which were found in the lumen; there was an uneven distribution of the nuclei of the epithelium, a formation of villi which shed cells from their apices into the lumen, a disappearance of the basement membrane etc. i. e. there was a destructive infiltration such as normally occurs in the second half of estrus after ovulation (ovulation did not occur in any animals in the 1st experiment). In 6 animals all these phenomena were weakly shown, and in one case were not shown at all (i. e. the uterine mucosa of this female was not in any way different from that of an unserviced female at the proestrus stage).

In the second experiment 11 females were serviced in the resting stage. Copulation caused destructive infiltrational changes in the uterine mucosa; these changes resembled those in the first experiment, but were very clearly shown in 8 out of the 11 animals.

In this experiment the formation of epithelial villi and the shedding of cells into the lumen was not well shown.

In the 3rd experiment, in 7 females serviced after estrus, destructive infiltration in the uterus was well shown in all animals. This may be partly due to the fact that after estrus the normal destructive phase is terminating. We may note, however, that copulation delays its completion.

In the 4th experiment, in females serviced 7-10 days after ovariectomy, the uterine reaction to insemination was very similar to that of experimental animals (Fig. 1).

The principle uterine reaction was shown in experiment 5, in females serviced 18 or more days after ovariectomy. In all the previous experiments, the changes in the mucosa were approximately uniform over the whole organ, but in this series the picture was very varied. Some parts remained intact, while others were more like the mucosa of females in the 2nd or 4th experiments, in which the reaction was very marked. In these places there had been a desquamation of the epithelium over a considerable expanse, the sperm had penetrated in a broad stream into the stroma and had infiltrated it right up to the muscle layer, the place where penetration had occurred being surrounded by a thick band of leukocytes, i. e., there had been a typical inflammatory reaction.
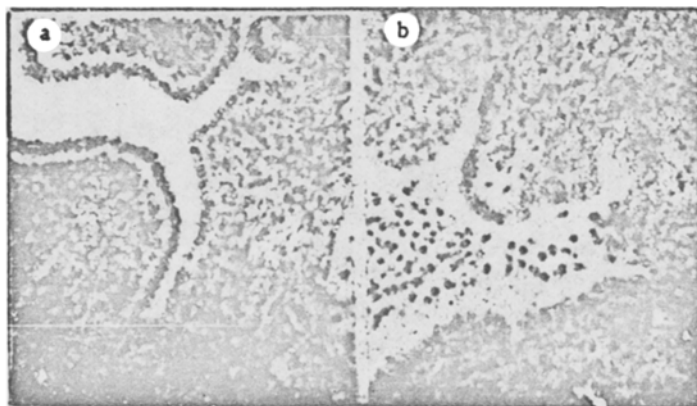

Fig. 1. Changes in the genital tract of the rat: a) Uterine wall of ovariectomized female. Even layer of short epithelium; b) the same 10 hours after copulation. Disorderly arrangement of the epithelial cells, desquamating in parts into the lumen. Leukocyte infiltration of the mucosa. Large number of leukocytes in uterine canal.

Investigation of the vagina in ovariectomized animals showed that here the stimulation of natural insemination, i. e., chiefly that of the vaginal plug, results in a considerable destructive infiltration (Fig. 2). It must be noted that all the above changes in the female genital tract do not occur if copulation is not completed, i. e., if there is no ejaculation and entry of sperm into the uterus. In order to find the effect of chronic stimulation, we also carried out experiments on 3 mice with copulation repeated at intervals of 4-5 days. Histological examination of the sexual organs of these females showed that the uterine wall had hypertrophied as compared with that of the control animals, the mucosa was thicker, and the epithelium better developed and richer in cells. The destructive infiltration following repeated insemination was more reminiscent of the reaction of ovariectomized females (absence of pathological signs and occurrence of the normal destructive phase of estrus, as for example the formation of epithelial villi, etc.).
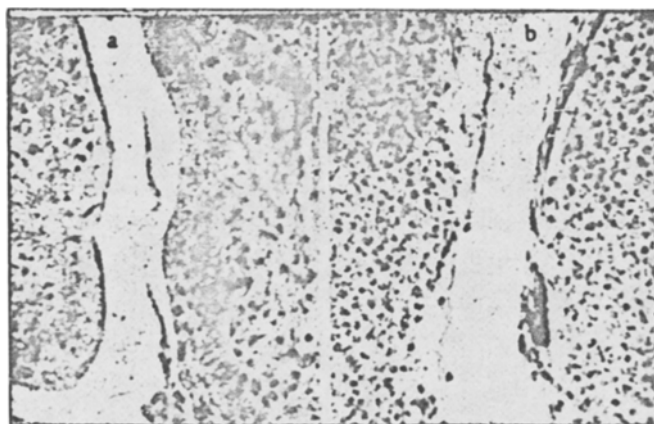

Fig. 2. a) Vaginal wall of ovariectomized female; b) the same,
a few hours after copulation. Marked infiltrational and destructive
changes.

Thus, our experiments show that natural insemination is a powerful physiological stimulus, which elicits destructive infiltrational changes in the genital tract of the female similar to those of the corresponding estrus phase. This phase, as had already been said, occurs in the second half of estrus and in the postestrus period, i.e., it occurs a few hours after natural insemination (which usually takes place in proestrus or at the beginning of estrus) and directly after ovulation, and therefore after fertilization.

The physiological basis of the normal estrus cycle is the complex neurohumoral mechanism of the sexual cycle. However, the results of the experiments here show that a very important and, biologically speaking, decisive part in eliciting the destructive phase in particular, is that of the powerful external stimulus of the act of insemination. Finally, the destructive changes obtained on inseminating nonestrus and ovariectomized females are not completely analogous to those of the destructive phase of normal estrus, though the differences are explicable in terms of the specific condition of the animals and of their reproductive organs. These differences are proportional to the degree of involution of the female genital system, and in animals which have been ovariectomized for a considerable time the reaction shows no qualitative differences. The act of insemination is no less important for the realization of the phase of proliferative repair. This is shown by the experiments with repeated copulation. It is generally accepted that the processes of destructive infiltration and proliferative repair are intimately associated.

Thus, natural insemination, considered as a biological stimulus, plays an important part in the realization of that complex cycle of changes in the uterus and vagina included in the term "estrus complex." We may suppose further that this complex arose originally as a reaction to insemination. In the course of phylogenesis, this reaction became separated from the original external stimulus through the formation of associated reflex pathways. Consequently, a self-contained neurohumoral estrus mechanism evolved, as a result of which the cyclic change which composed it can now take place without insemination occurring.

SUMMARY

The reaction of the genital tract of female mice in various stages of the estrus cycle and of ovariectomized females to coitus was investigated. Discharge of semen caused destructive-infiltrative changes in the uterus and vagina, which resembled the physiological destruction of the mucous membrane of these organs which was observed in the second half of estrus.

Similarity was the greatest in females in cases of copulation at the stage of rest or soon after ovariectomy (7-10 days).

Coitus plays an important part in the process of formation and maintenance of the cyclic destructive and proliferative changes of the mucous membrane of the genital tract of female mammals.

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* In Russian.
** Original Russian pagination. See C.B. translation.