

The Effect of Tubal Ligation on Ovarian Function in the Rat.* (19973)

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In the adult rat the periovarial sac is a double layer of mesothelium completely surrounding the ovary and containing within its cavity the opening of the oviduct. Below the ostium the sac is open for a short distance. Between the first and third days after birth the oviduct moves caudally and begins to coil, thus bringing the edges of the opening closer together. The peritoneal sac is found to be closed as early as the seventh day after birth although the exact time is variable. This study of Kellogg(1) confirms the statement of earlier workers(2-5). Alden(6) on the other hand, maintains that the ovarian sac in the rat has a peritoneal opening near the orifice of the oviduct. In order to prove his contention this investigator closed the opening with fine sutures. He found that 72 hours later the ovarian capsule was greatly distended with fluid. In addition the uterus was distended and vaginal smear demonstrated a proestrous state. By the end of 108 hours the distension had increased further and ovulation had occurred. Examination of the ovaries demonstrated fresh corpora lutea. Gumbrecht and Loeser(7) believe that the estrogenic hormone produced by the ovary reaches its target organ, the uterus, by way of the oviduct. They contend that this route carries the estrogen to the endometrium where it is absorbed. According to these investigators changes can be observed in the thyroid gland two weeks after hysterectomy, tubal ligation, or decapsulation of the ovaries in rats. They describe the histological picture of the thyroid as identical to that found in castrate animals.

In view of the interesting findings reported by earlier workers(1,4-6) we became interested in the possible role of the ovarian capsule in the maintenance of the normal estrous pattern of female rats.

Material and methods. A total of 20 young

female rats weighing between 150 and 180 g, of Sprague Dawley strain, bred and maintained in our laboratory, were used. Daily vaginal smears were followed in each experimental animal for a period of 2 to 3 weeks to establish normal estrous rhythm in each animal. Any rat which showed aberrations of the normal estrous cycle was discarded. Following this control period the animals were operated on under ether anesthesia and both oviducts were ligated with a single cotton suture at the junction of the uterine horn and the fallopian tube. The daily vaginal smears were continued.

In all experimental animals following tubal ligation the pattern of the estrous cycle changed markedly. Seven to 10 days after surgery all animals went into prolonged periods of estrus which were only occasionally interrupted for periods of from 1 to 3 days of proestrous after which they returned to the extremely heavy estrous smear. This pattern

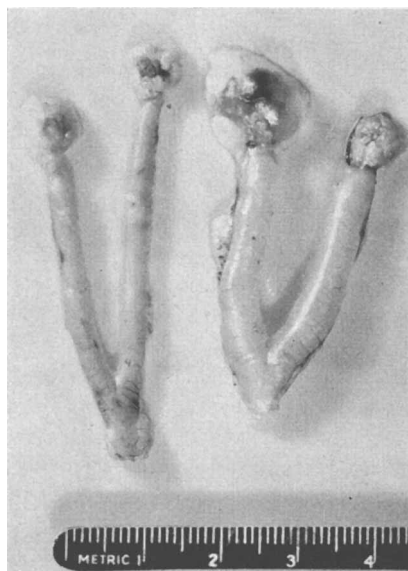


FIG. 1. Normal (left) and tubal ligated (right) rat ovaries and uteri. The capsule has been removed from the right ovary of the ligated specimen.

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FIG. 1-a. Overdistended ovarian sac. Hyperemia of ovary, tube, and uterine horn.

was followed in some animals for as long as 3 months.

When the animals were sacrificed the ovarian capsules were found to be grossly distended (1.5 to 2 cm in diameter). The fallopian tubes were somewhat distended and the uterine horns demonstrated estrogenic effect. There was marked hyperemia of the ovary, oviduct and uterus. (Fig. 1, Fig. 1-a)

The fluid from the ovarian capsule was collected and injected into castrated female rats. These animals showed estrogenic activity of the fluid as demonstrated by estrous smears.

When the experimental animals (with tubal ligation) were castrated the vaginal smear reverted to typical diestrous picture on the second or third day postoperatively.

In a second group of rats after the typical pattern of estrus following tubal ligation was well established the animals were again anesthetized and the distended capsule carefully stripped from the ovary. In every case following this procedure the animal gradually returned to the typical estrous cycles of the control animals in 4 to 7 days.

The histological examination of the ovaries, uteri, thyroids, and pituitaries of the experimental animals was extremely interesting. Ovaries were examined after 30 and 90 days following tubal ligation. In the first group (30 days after tubal ligation) the ovaries appear markedly active. Graafian follicles in various stages of development are seen. The cells of the cortex stain deeply. Many mitotic figures are present in the graafian follicles. No new corpora lutea are evident. The medullary portion of the ovary appears unchanged. (Fig. 2)

On the other hand 90 days after tubal ligation marked evidences of degeneration are seen in the ovaries. The cortex contains only a few graafian follicles and the remnants of



FIG. 2. Graafian follicles in various stages. Many mitotic figures in the follicles. No new corpus luteum.



FIG. 3. Large cystic spaces in the cortex and medulla. Increased connective tissue. Remnants of old corpora lutea.

old corpora lutea. The cells are vacuolated and decreased in size. The amount of connective tissue has increased and dominates the field. Large cystic spaces are present in the cortex and medulla (Fig. 3).

In those cases where the ovarian capsule was removed 60 days following tubal ligation the ovaries demonstrated typically normal histology.

The uterus shows histological changes as well. The muscle fibers, the connective tissue elements, and the endometrium are edematous. The glands are simple tubes without tortuosity, characteristic of estrogenic stimulation (Fig. 4). The vascular structures are distended. In the animals where tubal ligation was permitted to remain for 90 days the histology of the uterus was similar to that of castrate animals in spite of continuous estrogenic response of the vaginal mucosa.

The thyroids appeared enlarged but we were unable to observe the castration changes described by Gumbrecht and Loeser.

Discussion. In view of our findings it is difficult to understand the observation of

Alden(6) who states that the ovarian sac has a peritoneal opening near the orifice of the oviduct. One would expect that distension of the sac would not occur by simple ligation of the oviduct at the junction with the uterine cornu. This in no way interferes with the capsule of the ovary.

It is also difficult to understand the findings of Gumbrecht and Loeser(7) who state that the "capsule serves to force the follicular fluid, liberated at ovulation to pass into the uterus where the estrogen will act upon the uterus." They state further that the follicle hormone avoiding the circulation is able, and only in this manner, to act as estrogenic hormone.

While it is altogether feasible to suppose that some estrogen produced by the ovary reaches the uterus in this manner, ligation of the oviduct with resultant capsular distension and uterine stimulation proves that this is not the only route of estrogens from the ovary to the uterus. If one is to postulate a function of the capsular fluid it is logical to presume that it may serve as an aid in the transportation of the liberated ovum to the uterus.

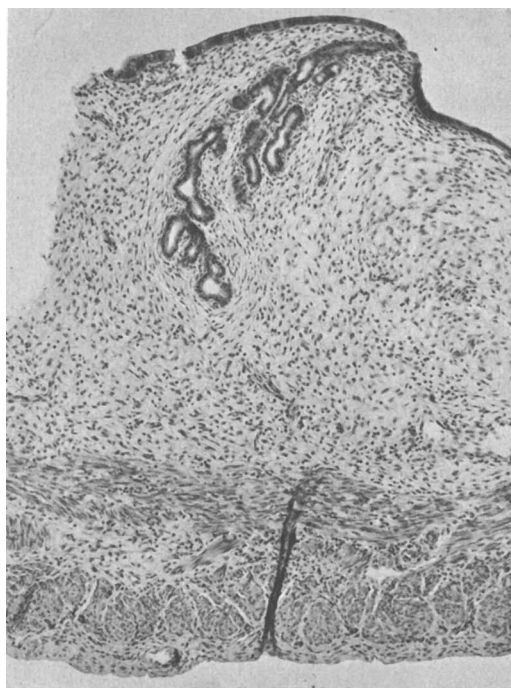


FIG. 4. Edematous uterine wall. Estrogenic response in the glands of endometrium.

The observation of prolonged estrus in animals in which the fallopian tubes were ligated is especially interesting. The manner in which this is brought about is under study at the present time. Whether the apparent increase in estrogen production is the result of increased FSH from the anterior pituitary is to be determined. The lack of corpora lutea formation is also unusual.

The cystic changes that appear in the ovaries of animals 90 days after tubal ligation can probably be best explained on the basis of an inadequate blood supply. As the fluid accumulates in the ovarian sac increased pressure induces ischemia of the tissues with resultant degenerative changes.

Summary and conclusions. 1. Young healthy female rats with normal estrous cycles when treated by simple bilateral ligation of the oviducts proximal to the uterus show prolonged periods of estrus. 2. When the distended capsule produced by this procedure is removed the animals resume normal estrous cycles. 3. The fluid obtained from the capsule

contains estrogenic hormone as demonstrated by estrous smears in spayed female rats. 4. The noteworthy histological changes in the ovaries after 30 days of tubal ligation are marked follicle stimulation and the absence of new corpora lutea. After 90 days following ligation the ovaries become cystic, probably due to an inadequacy of the blood supply.

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Inhibition of the Hooker-Forbes Response by Aminopterin.* (19974)

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Numerous papers have appeared in the literature concerning the effects of folic acid antagonists on estrogen and androgen-induced growth in the reproductive tracts of laboratory animals(1-14). Until recently, however, very little experimental work has been directed toward ascertaining the influence of folic acid antagonists (F.A.A.) on the action of progesterone. It was mentioned in a preliminary report from this laboratory(15) that one of the F.A.A. (aminopterin;† 4-amino pteroylglutamic acid) would inhibit the effects of

progesterone on the stromal connective tissue cells in the uteri of ovariectomized mice. This paper presents a more detailed study of the effects of aminopterin on the Hooker-Forbes reaction(16), a progestational response elicited by small amounts of progesterone in ligated segments of the uteri of ovariectomized mice.

Materials and methods. A total of 53 Swiss albino mice, weighing 20 to 23 g, were used for these experiments. The mice were maintained in a warm room, and fed the standard Rockland mouse diet. They were ovariectomized via the dorsolumbar route, and 16 days after the operation were given a single intra-uterine injection. The folic acid antagonist (aminopterin), estradiol 17-beta and progesterone were dissolved alone or in com-

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