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 intrauterine injection. The folic acid antagonist (aminopterin), estradiol 17-beta and progesterone were dissolved alone or in com-

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† Aminopterin (4-amino pteroylglutamic acid) was obtained through the courtesy of Lederle Laboratories, Pearl River, N. Y.

TABLE I. Effect of Progesterone, Aminopterin, and a Combination of Both on the Hooker and Forbes Assay for Progesterone in the Uterus of the Mouse.*

Treatment μg	Treatment μg	Results
9 P†‡		+
20 A		—
40 A		—
50 A		—
10 A + 9 P§		+
20 A + 9 P		+
30 A + 9 P		+
40 A + 9 P		—
50 A + 9 P		—

* Three to 5 mice per experimental group.
† Total amount inj. .0006 cc.
‡ P = Progesterone. A = Aminopterin.
§ Aminopterin and progesterone combined in amounts indicated and dissolved in 1 cc of physiological saline. Total amount inj., .0006 cc.

binations in an aqueous medium. Forty-eight hours after intra-uterine injection, the mice were killed and the uteri were carefully dissected out, fixed in Lavdowsky's fluid, embedded in paraffin, sectioned at 7 microns, and stained lightly with hematoxylin and eosin. The slides were then examined, and evaluated in terms of a positive (+) or negative (—) Hooker-Forbes response.

Results and discussion. A positive Hooker-Forbes response invariably results when 0.0006 cc of a solution containing 9 μg progesterone per cc is injected into an isolated segment of the uterus of a castrated mouse. However, when the same dosage (0.0006 cc) of a similar solution of progesterone (9 μg/cc) is given to which 40 to 50 μg aminopterin per cc has been added, the action of progesterone is completely inhibited. In similar experiments in which 10 to 30 μg aminopterin per cc was combined with the stock solution of progesterone, there was no apparent interference with the Hooker-Forbes reaction (Table I). In those mice in which the reaction was inhibited, the stromal nuclei had the same appearance usually found in ovariectomized, non-treated mice. Such nuclei were irregularly shaped, dense, and pyknotic in contrast to the oval shaped nuclei containing fine chromatin granules and distinct nucleoli in the non-inhibited progesterone-treated mice. Inhibitory doses of aminopterin when injected alone did not repair the castration atrophy in the stromal nuclei.

These experiments show that aminopterin is quite effective in inhibiting the action of progesterone on the uterine stromal nuclei. Likewise, in other experiments(14), it has been demonstrated that 4-amino PGA inhibits the action of estrogen on the uterus with equal facility. However, it seemed possible that aminopterin in the presence of estrogen and progesterone might show a preference for one or the other steroid in the inhibitory action. Previously, workers in this laboratory(17) reported that estrogen and progesterone in a ratio of 1:200 gave a negative Hooker-Forbes response. This experiment was repeated and confirmed. In addition to the 1:200 ratio of estrogen and progesterone (*i.e.*, 0.0006 cc of a solution containing 0.025 μg estradiol 17-b and 5 μg progesterone per cc), 30 to 50 μg of aminopterin per cc were administered concurrently. Both the response of the stromal nuclei of the endometrium to progesterone and the increase in height of the epithelium of the uterine lumen and glands, characteristic of estrogenic action, were completely inhibited. These results suggest the possibility of a common requirement for the action of both of these steroids (Table II).

The clear-cut inhibition of the Hooker-Forbes reaction obtained by the administration of 0.0006 cc of a solution containing 40-50 μg aminopterin plus 9 μg progesterone per cc strongly suggests that aminopterin is a potent inhibitor of progesterone as well as estrogen. It seems quite probable that the inhibition produced by aminopterin has a direct effect on the action of estrogen and progesterone, and is not due to the toxicity of

TABLE II. Effect of Combinations of Estradiol, Progesterone, and Aminopterin on the Hooker-Forbes Reaction.*

Treatment	Results
5 μg progesterone†	+
Estradiol:progesterone (1:200)	—
E:P (1:200) + 30 μg A‡	—
" + 40 A	—
" + 50 A	—

* Three to 5 mice per experimental group.
† All substances for a particular treatment were combined in 1 cc of physiological saline, and the amount inj. was .0006 cc.
‡ A = Aminopterin.

the inhibiting compound. This is in agreement with the observations of Velardo and Hisaw(18) on the inhibition of decidual development by 4-amino PGA in rats. The present state of our knowledge of the action of aminopterin indicates that it interferes in some way with the enzyme systems associated with the physiological activity of estrogen and progesterone(19-25).

Summary. The response of the uteri of ovariectomized mice to a total amount of 0.0054 μg progesterone was inhibited by the simultaneous administration of 0.0240 to 0.030 μg 4-amino pteroylglutamic acid. Aminopterin (4-amino PGA) showed no selective inhibition for estrogen or progesterone when combined with these two steroids, but inhibited both of these compounds when injected within a ligated segment of the uterus of ovariectomized mice according to the method of Hooker and Forbes.

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Rabies Street Virus Strains in the Syrian Hamster and in the Swiss Albino Mouse. (19975)

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Specific inclusion bodies present in the central nervous systems of animals dying from rabies are called "Negri bodies"(1,2). These are most frequently demonstrated within, but are revealed occasionally outside the nerve cells. A definite diagnosis of rabies is made upon demonstration of these bodies in stained smears. These inclusions are eosinophilic bodies, usually spherical, but of other shapes, varying from 1 to 30 μ in size. The inner structure shows basophilic granules. At present the animal most frequently used for the diagnosis of rabies is the mouse. A positive diagnosis is made on the development of symptoms of rabies in the mouse and the presence of Negri bodies in the mouse brain.