

there is an inhibition or depression of the formation of small follicles which is a direct effect of stilbestrol on the ovary. Table I, i shows that, after a slight initial depression during the first 4 days of injection, there is an increase in the number of normal antrum-containing follicles. This increase indicates that follicular development still proceeds from the small to the antrum-containing stage. This again differs from the results of hypophysectomy where there is a complete absence of normal antrum-containing follicles within 4 days after operation, and indicates that FSH is not completely blocked by massive doses of stilbestrol. Table I, j shows that there is an initial decrease in the number of

atretic antrum-containing follicles, followed by a marked increase towards the end of the experimental period. This indicates that the follicle-stimulating function is depressed to some extent. The delay in the appearance of increased atresia argues against a direct effect.

*Summary.* These results support previous evidence that the ovarian structures which normally secrete estrogen, *i.e.*, antrum-containing follicles and corpora lutea, are affected when the estrogen level is raised, presumably via the anterior hypophysis. However, the suppression of formation of new small follicles is evident as a direct effect of stilbestrol on the ovary.

Received June 20, 1949. P.S.E.B.M., 1949, 71.

### 17250. Male Mice Tolerate Dosages of Pteroylglutamic Acid\* Lethal to Females.

ALFRED TAYLOR AND NELL CARMICHAEL.

*From the Biochemical Institute, University of Texas, and the Clayton Foundation for Research, Austin, Texas.*

Female mice are much more susceptible than males to high dosages of pteroylglutamic acid (folic acid). Male mice easily tolerate amounts of this material lethal to every female injected. Females receiving sublethal levels of the compound lose more weight and are slower to recover than comparably treated males.

Harned<sup>1</sup> and associates have investigated the pharmacology of folic acid. They reported this compound to be comparatively innocuous at levels much above the therapeutic range.

When 100 to 400 mg per kg were administered intravenously, acute toxicity was observed in mice, rats, rabbits, and guinea pigs. Death was apparently due to obstruction of renal tubules as a result of the precipitation of folic acid. The mouse and the rat were more resistant than the guinea pig and the rabbit.

\* Pteroylglutamic acid for this research was furnished by Lederle Laboratories, Inc.

<sup>1</sup> Harned, B. K., Cunningham, R. W., Smith, H. D., and Clark, M. C., *Ann. N. Y. Acad. Sci.*, 1946, 48, 289.

There is no mention in the paper of a sex difference in the response of the animals to the material injected.

*Experimental.* More than 400 mice of the dba strain were used in these experiments.

Folic acid was administered both in the form of a saline suspension (0.85%) and dissolved in a solution of sodium bicarbonate (3%). The saline suspension was most frequently used. The various amounts given were diluted so that each animal received 0.1 to 0.2 cc per injection. The dosages given were based on a 25 g mouse unless otherwise stated.

Healthy mature dba male and female mice were subjected to single subdermal injections of 1 to 40 mg (saline suspension) of folic acid to test the effect of massive doses on mortality. The results are summarized in Table I.

Autopsy disclosed the usual precipitation of folic acid in the renal tubules as indicated by the yellowish color of the kidneys. The spleen was reduced in size. Weight averages

TABLE I.  
Effect of a Single Injection of Folic Acid on Mortality of Male and Female dba Mice.

| Dosage<br>(mg per 25 g mouse) | Male         |          | Female       |          |
|-------------------------------|--------------|----------|--------------|----------|
|                               | No. injected | No. dead | No. injected | No. dead |
| 1                             | 3            | 0        | 3            | 0        |
| 3                             | 3            | 0        | 3            | 0        |
| 5                             | 10           | 1        | 15           | 3        |
| 10                            | 8            | 2        | 11           | 9        |
| 15                            | 7            | 0        | 7            | 7        |
| 20                            | 8            | 0        | 8            | 8        |
| 40                            | 4            | 0        | 4            | 4        |

of spleens from female mice receiving 10 mg of folic acid and spleens from control females showed an average reduction of 40% in the spleen weights of the experimental mice. The visceral effects in the male were similar to those observed in the female.

Male and female mice of the same initial body weight were given sublethal injections of folic acid and the effect on the body weight recorded over a period of time.

One group consisted of 20 male and 20 female mice not yet fully mature so that the initial body weights could be exactly matched. Each mouse received a single injection of 5 mg folic acid. The females showed a 10% loss in body weight and had not completely recovered 12 days after the administration of the compound, while the males showed only slight loss of weight followed by rapid recovery and gain in weight.

In other tests mature male and female mice manifested the same sex difference in their response to sublethal injections of folic acid.

In one experiment 6-week-old male and female mice were given single injections of 5 mg folic acid. The males continued to gain

weight but more slowly than normal. They gained an average of 3 g in the week following the treatment. The females, one week after the injection weighed an average of 1 g less than their initial weight indicating they were seriously affected.

*Discussion.* Why the female should be so much more susceptible than the male to high dosages of folic acid has not been determined.

So far as could be discovered no other compound has ever been reported which manifests such a sex difference in its pharmacology.

*Summary and conclusion.* Male mice are much more resistant than female mice to injections of high dosages of folic acid.

Male mice were only slightly affected by single subdermal injections of 5 mg of folic acid. Female mice receiving the same dosage averaged a 10% loss in body weight followed by a slow recovery.

There were no deaths in a group of male mice receiving 15 to 40 mg in a single injection of folic acid. The same dosages administered to female mice were rapidly lethal in every instance.

Received June 28, 1949. P.S.E.B.M., 1949, 71.