

17249. Reactions of Normal Ovaries to Injections of Stilbestrol.*

G. GORDON ROBERTSON. (Introduced by Carroll A. Handley.)

From the Department of Anatomy, Baylor University College of Medicine, Houston, Texas.

It is a common belief that injected hormones do not stimulate the organs which normally produce them. While injected estrogens may have no direct stimulatory effect on ovarian follicles of normal adult animals, they do produce certain ovarian changes either by direct action on the ovaries, or indirectly via the anterior pituitary gland.^{1,2} The consensus is that, if the injected doses are of sufficient potency, there will be (1) luteinization,^{3,5} and (2) inhibition or depression of follicular development,^{2,6,7} and that these effects are secondary to pituitary changes produced by increased estrogen. It is probable that FSH normally stimulates medium-sized follicles to develop fully to the ovulating stage. If the estrogen level goes over a certain point, the production of FSH is inhibited or depressed, thus resulting in failure of the development of ovulating follicles. At the same time, LH is liberated, either as a result of inhibition of FSH or due to the direct stimulatory effect of increased estrogen, and LH stimulates luteal formation in the ovary. There is the possibility that luteinization is an effect of increased estrogens acting directly on the ovaries, but assays of pituitary

glands after estrogenic treatment show an increased potency of luteinizing function.⁸

Materials and methods. Follicular development and luteinization in the ovaries of normal adult rats were studied after intraperitoneal injections of large doses of the synthetic estrogen, diethylstilbestrol dipropionate ("Estrobene DP"). Inbred Sprague-Dawley rats were used, and the work was done on 100-day-old virgin females. The ovaries of 11 control rats were removed at the 5 stages of the estrous cycle and were serially sectioned. In the experimental group, consisting of 15 rats, 5 mg of stilbestrol were injected intraperitoneally once per day, ranging from 1 to 12 injections. All of the injections were begun at diestrus. The ovaries were removed 24 hours after the last scheduled injection, were fixed and serially sectioned. From the serial sections of the control and experimental groups the following determinations were made: (1) the total volumes of the ovaries were determined by planimeter measurements of serial projection drawings; (2) the total volumes of the luteal tissue were measured by the same methods, and the percentage luteinization determined in each case; (3) the follicles were counted and divided into 4 categories: normal and atretic small follicles, and normal and atretic follicles in which an antrum had definitely formed.

Results. In the results, which are summarized in Table I, the animals are divided into 4 groups, and the average figures are given for each group. Group A consists of the 11 control animals in the various stages of the normal 4-day estrous cycle. Groups B, C, and D consist of 15 animals which received daily injections of 5 mg of diethylstilbestrol over a period ranging from 1 to 12 days. In Group B, the animals received 5 to 20 mg in the 4-day period ranging from 1 to 4 days; in Group C, 25 to 40 mg in the

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¹ Kunde, M. M., D'Amour, F. E., Gustavson, R. G., and Carlson, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 122.

² Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

³ Fevold, H. L., Hisaw, F. L., and Greep, R., *Am. J. Physiol.*, 1935, **114**, 508.

⁴ Desclin, L., *Compt. rend. Soc. de biol.*, 1935, **120**, 526.

⁵ Meyer, R. K., and Hertz, R., *Am. J. Physiol.*, 1937, **120**, 232.

⁶ Allen, E., and Diddle, A. W., *Am. J. Obst. and Gynec.*, 1935, **29**, 83.

⁷ Bullough, W. S., *J. Endocrinology*, 1943, **3**, 235.

⁸ Lane, C. E., *Am. J. Physiol.*, 1934, **110**, 681.

TABLE I.
Summary of Effects of Stilbestrol on the Ovary.

Determinations	Group A, control, avg	Group B, 1-4 day stilbestrol, avg	Group C, 5-8 day stilbestrol, avg	Group D, 9-12 day stilbestrol, avg
a. Volume of ovaries in 1000 planimeter units	46.6	39.8	44.9	56.9
b. Volume of corpora lutea in 1000 planimeter units	20.7	16.1	21.2	30.4
c. % luteinization	44.2	40.1	45.8	53.0
d. Total No. of follicles	435	292	235	281
e. Total No. of normal follicles	334	229	187	190
f. Total No. of atretic follicles	101	62	48	91
g. No. of normal small follicles	259	159	97	75
h. No. of atretic small follicles	61	36	18	24
i. No. of normal antrum-containing follicles	75	70	80	115
j. No. of atretic antrum-containing follicles	40	26	30	67

4-day period ranging from 5 to 8 days; and in Group D, 45 to 60 mg in the 4-day period ranging from 9 to 12 days.

With progressive injections of stilbestrol there is an initial decrease in ovarian size during the first 6 days, followed by a marked increase over the normal size (Table I, a). Similarly with luteal tissue (Table I, b), there is an initial decrease followed by a marked increase in luteinization, which is directly proportional to the increase in ovarian size. The degree of luteinization is shown in Table I, c. During the stilbestrol injections there is an initial 4-day lag followed by a marked increase in the degree of luteinization. The initial lag may be due to a natural hypophyseal-gonadal lag, which has been indicated by the 2-4-day delay in the effect of hypophysectomy on ovaries.⁹

Following treatment with stilbestrol there is a significant decrease in the total number of follicles from the second day on (Table I, d). This consists of a decrease in the number of both atretic and normal follicles (Table I, e,f), with some recovery in the number of atretic follicles towards the end of the experimental period. If there is a

complete physiological block of FSH after injections of massive doses of stilbestrol, one would expect the follicular picture to resemble that after hypophysectomy, except for any direct effects stilbestrol may have on the ovaries. Smith⁹ has described the follicular picture after hypophysectomy in the rat as follows: (1) all medium-sized and large follicles (*i.e.*, all antrum-containing follicles) become atretic within 4 days or less, and formation of normal follicles in this group is terminated; (2) small follicles continue to develop, but become atretic before antrum formation. In an attempt to analyse the effects of large doses of stilbestrol, the follicles in these two categories were counted and the number of normal and atretic follicles determined in each.

It is evident that there is a continuous and rapid decline in number of normal small follicles with progressive stilbestrol injections (Table I, g). Similarly, as shown in Table I, h, there is a decrease in the number of atretic small follicles. This decrease in the number of small follicles indicates that in our series

⁹ Smith, Philip E., *Am. J. Anat.*, 1930, **45**, 205.

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.

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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.

Harned¹ 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.

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¹ 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