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Mammary Carcinomas in Mice Following Oral Administration of Stilbestrol.

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(Introduced by Carl Voegtlin)

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Mammary carcinomas are readily induced by long-continued administrations of large doses of estrogens to mice of susceptible strains. In the numerous investigations[1] on the subject, the subcutaneous route of injection has been used almost exclusively. The induction of breast tumors following oral administration of estrogens has not been reported. The natural estrogens lose most of their estrogenic potency when given by mouth,[2] whereas the synthetic product, stilbestrol (4:4′-dihydroxy-α:β-diethylstilbene) is effective orally,[2] and was used in this experiment.

Forty-one male mice of strain C_3H, raised in this laboratory, were used. Spontaneous mammary tumors develop in practically 100% of the virgin females of this line, but do not occur in the males.[3] The mice were maintained on a standard diet and an unlimited supply of water. They were about 2 months old and weighed from 21 to 23 g when the experiment was initiated.

Stilbestrol* was dissolved in sesame oil, and was fed to the mice by means of a curved metal tube passed into the esophagus. The animals were divided into 4 groups: (I) Eleven were given 0.125 mg of stilbestrol dissolved in 0.1 cc of sesame oil twice a week for 17 weeks, for a total dose of 4.25 mg of the compound. (II) Ten received 0.375 mg of stilbestrol in 0.1 cc of sesame oil twice a week for 17 weeks for a total of 12.75 mg. (III) Ten were fed 0.1 cc of sesame oil twice a week for 17 weeks. (IV) Ten were kept as untreated controls.

The first effect noted was the marked retardation in the gain in weight of the mice which were ingesting stilbestrol (Figure 1). This was not due to loss of appetite, since the consumption of food per gram of body weight was identical in similar groups of mice in which

1 Gardner, W. U., *Arch. Path.*, 1939, **27**, 138.

2 Leighty, J. A., and Wick, H. J., *Endocrinology*, 1939, **25**, 597.

3 Andervont, H. B., and McEleney, W. J., *Pub. Health Rep.*, 1938, **53**, 777.

* We are indebted to Merck and Co. for the compound.

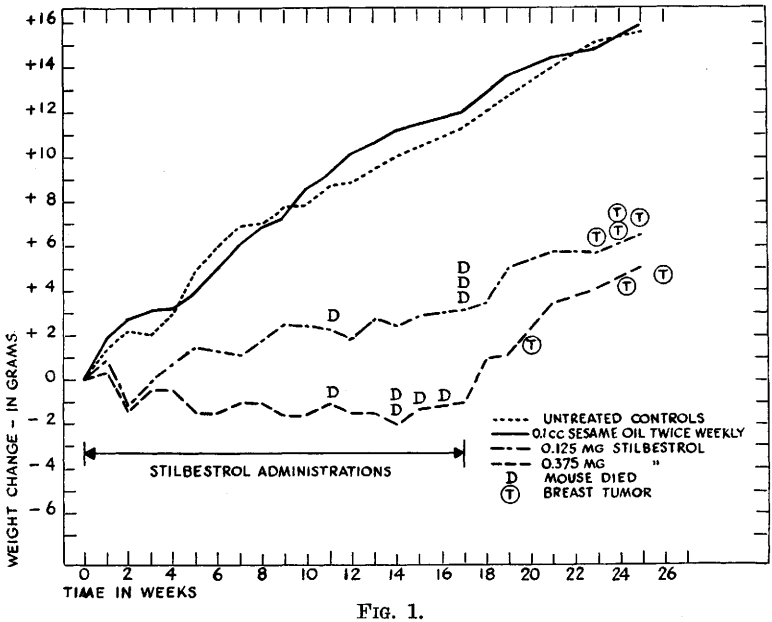

FIG. 1.
Response of male C_3H strain mice to bi-weekly oral administration of stilbestrol.

the food intake was measured for 3 months. The retardation in weight also occurs when the compound is given subcutaneously.[4]

Four of the 11 mice of Group I, and 5 mice of Group II died 11 to 17 weeks after the initial administration of stilbestrol. All showed marked atrophy of the spleen, testes, and seminal vesicles. These atrophic changes were not observed in the mice which developed breast tumors 6 weeks or longer after the cessation of stilbestrol feeding. Presumably such lesions are at least partially reversible if administration of estrogens is discontinued.[5] There was a high incidence of dilatation of the urinary bladder, but death was not attributed to this finding. There were no consistent changes in the gastroenteric tract. No evidence of hepatic damage was seen in these mice, nor in mice of strains C_3H, C_{57} black, C or A which received 5 mg or more of stilbestrol subcutaneously and which have died in the course of experiments now in progress.

Breast tumors, recognized by palpation, began to appear 20 weeks after the initial administration of stilbestrol. Seven mice which received 4.25 mg of the estrogen were alive at this time; of these, 4 have developed tumors, in 23, 24, 24, and 25 weeks. Five mice which

[4] Gaarenstroom, J. H., and Levie, L. H., *J. Endocrinol.*, 1939, **1**, 420.
[5] Shimkin, M. B., and Grady, H. G., *J. Nat. Cancer Inst.*, 1940, **1**, 119.

ingested 12.75 mg of stilbestrol were alive at 20 weeks; of these, 3 have developed tumors, in 20, 24, and 26 weeks.

All 7 tumors were adenocarcinomas, of the same histologic type as the spontaneous tumors in the females of the strain and the tumors in the males following the subcutaneous injection of estrone or stilbestrol.[5]

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Effect of Anti-Placenta Serum on Development of Foetus in the Pregnant Rat.

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This paper reports a series of experiments designed to determine the effect of rabbit anti-rat-placenta serum on pregnancy in the rat. The specificity of the results produced by the anti-placenta serum was controlled by the use of normal rabbit serum, rabbit anti-follutein and anti-pituitary serums, rabbit anti-rat-serum serum, and rabbit anti-rat-whole-blood serum. Another group of pregnant animals received no treatment. The experiments were carried out over a period of 3 years in 2 strains of rats.

Preparation of Antiserums. The method of preparing the rabbit anti-rat-placenta serum was as follows: Pregnant rats were sacrificed one day before term, the placentae were washed in saline, weighed, ground with sand and suspended in saline. The average yield of placental tissue from one rat was 4.5 g; this material was divided into 10 parts and injected intraperitoneally into each of 2 rabbits on 5 successive days. After a rest period of one week the animals were given a second, third and fourth course of immunizing injections and were bled out 10 days later. The serum was inactivated at 56°C for 30 minutes and stored without preservative.

Seven rabbit anti-rat-placenta serums were prepared; the last 3 serums were obtained by immunization with placentae from rats which had previously been perfused with 2 liters of saline, in the manner described by Smadel.[1] The results obtained with all 7 serums were identical.

Two rabbit anti-hormone serums were furnished by Dr. S. C.

[1] Smadel, J. E., *J. Exp. Med.*, 1936, **64**, 921.