17. Pulvertaft, R. J. V., Davies, J. R., Weiss, L., Wilkinson, J. H., *J. Path. and Bact.*, 1959, v77, 19.

18. Jones, G. E. S., Gey, J. C., Gey, M. K., *Bull. J. H. H.*, 1943, v72, 26.

19. Waltz, H. K., Tullner, W. W., Evans, V. J., Hertz, R., Earle, W. R., *J. Nat. Cancer Inst.*, 1954, v14, 1173.

20. Guillemin, R., Rosenberg, B., *Endocrinology*, 1955, v57, 599.

21. Schaberg, A., *Proceedings*, Series 60, No. 4, 1957, pp. 463-470 Koninkl. Nederl. Akademie van Wetenschappen, Amsterdam.

22. Ezrin, C., Swanson, H. E., Humphrey, J. G., Dawson, J. W., Wilson, W. D., *J. Clin. Endocrinol.*, 1958, v18, 917.

23. Stulberg, C. S., Soule, H. D., Berman, L., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 428.

24. Diczfalusy, E., Hogberg, B., Westman, A., *Acta endocrinol.*, 1950, v5, 226.

25. Raben, M. S., Westermeyer, V. W., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 550.

26. ———, *ibid.*, 1952, v80, 83.

27. Raben, M. S., *Science*, 1957, v125, 883.

28. Hsu, T. C., Klatt, O., *J. Nat. Cancer Inst.*, 1958, v21, 437.

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Mechanism of Tumor Inhibition by Estrogen.*† (25266)

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It has been shown that estrogen enhances the development of immunity against transplantable tumors in mice(1) and the postulate has been advanced that inhibition of growth of transplantable tumors during pregnancy might be due to hyperestrogenemia(2). The administration of estrogen also increases production of endometrial secretions (uterone)(3,4) and enhances biosynthesis in the endometrium(5). Since endometrial secretions were shown to inhibit growth of transplanted tumors in mice under certain conditions(6), it was logical to hypothesize that some of the tumor-inhibiting effects observed during induced or physiological hyperestrogenemia might be mediated through the uterus (6). The present paper deals with a study to determine the possible role of the uterus in tumor inhibition by estrogens.

Materials and methods. Forty immature AKD2F_1 (AKS x DBA/2) female mice, obtained from the Jackson Memorial Laboratory, housed in air-conditioned animal rooms throughout the experiment, were fed Purina Laboratory Chow Checkers and tap water *ad libitum*. The animals were divided into 4 groups of 10 individuals, and surgery was performed on all 4 groups (under nembutal anesthesia) according to the protocol shown in Table I. At operation, estradiol benzoate pellets of known weight were implanted subcutaneously in Groups II and IV. All animals were given achromycin in their drinking water for 5 days following surgery, and on the eleventh postoperative day tumor transplants were made. Sarcoma 1 cells from an AKS female host (obtained from Jackson Memorial Lab.) were suspended in chilled Ringer's solution according to the cytosieve method of Snell(7) and adjusted to a high cell concentration (17%) as measured by means of a Wintrobe hematocrit tube. All animals were injected subcutaneously with 0.2 cc of the same tumor suspension. The order of the injections was arranged so that an identical num-

TABLE I.

Groups of 10 mice	Surgical condition	Treatment
I	Intact (sham operation)	
II	*Idem*	Subcut. estradiol benzoate pellet
III	Hysterectomy & castration	
IV	*Idem*	*Idem*

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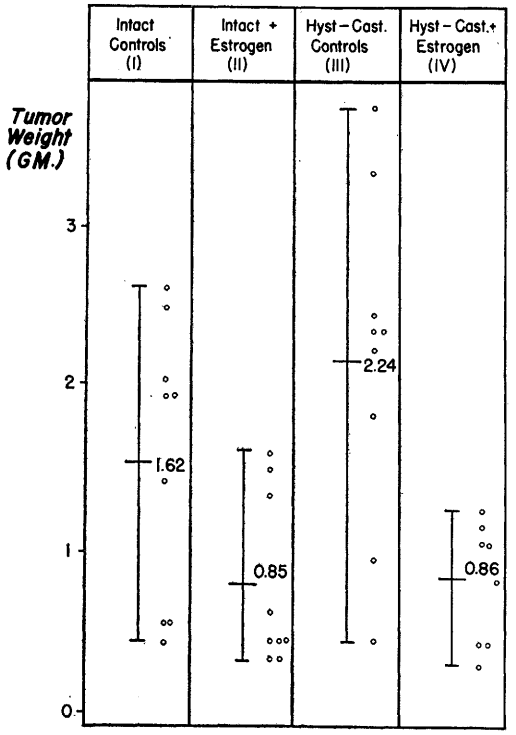

FIG. 1. Weights of tumors (avg and range) in estrogen-treated animals and controls.

ber of animals in each group received cells of similar suspension age. The animals were sacrificed by cervical dislocation on the 10th day following injection of the tumor. The tumors were dissected free of surrounding tissue, weighed, and placed in Bouin's fixative for sectioning and staining. The uteri (where present) were also excised and prepared for histological examination. The estrogen absorbed was determined by subtracting the dry weight of the pellets recovered at the end of the experiment from the initial pellet weight. This dose averaged 0.23 mg of estradiol benzoate per day per mouse.

Results. The results in terms of tumor weights are shown in Fig. 1. Body weights of all animals increased on the average during the experiment, and there were no significant differences between the groups.

In estrogen-treated animals, regardless of presence or absence of the uterus, tumor weights were significantly smaller than in untreated controls.

The reduction of tumor weights in estrogen-treated hysterectomized castrates (Group IV) was statistically significant when compared with their controls (Group III) (p. 0.005), as was the reduction in estrogen-treated, intact animals (Group II compared with Group I) (p. 0.025).

Tumors from both estrogen-treated and untreated animals showed variable amounts of necrosis and hemorrhage. Tumors from the estrogen-treated animals appeared to have thicker fibrous capsules and more active stroma than did those from animals not receiving estrogen.

The tumor growth in untreated, hysterectomized castrates (Group III) seemed to be greater than in untreated, intact animals (Group I), although this difference was not statistically significant (p. 0.1).

The uteri of the estrogen-treated intact animals showed evidence of marked estrogenic effects, with pronounced glandular changes. Immature uteri were found in the intact, untreated animals.

Discussion. It appears that inhibition by estradiol benzoate of transplanted Sarcoma 1 in AKD2F_1 hybrid female mice is independent of the uterus, since this inhibition was exactly equal in hysterectomized castrated mice and in intact animals. It would therefore seem that the tumor-inhibiting effect of pregnancy(2) and of endometrial secretions (6) are due to different mechanisms. Since uterone is devoid of estrogenic effects (unpublished), its tumor-inhibiting mechanism remains to be elucidated.

Summary. Immature AKD2F_1 mice were examined for possible estrogenic inhibition of Sarcoma 1. Large doses of estradiol benzoate inhibited growth of tumor in intact animals and in hysterectomized castrates. It was concluded that this estrogenic effect can be achieved in absence of uterus, and that the tumor-inhibitory properties of endometrial secretions act by a mechanism different from that of estrogen.

1. Nathanson, I. T., Salter, W. T., *Arch. Path.,* 1939, v27, 828.

2. Homburger, F., Tregier, A., *Cancer Res.,* 1954, v14, 490.

3. Nilsson, O., *J. Ultrastructure Research,* 1959, v2, 331.

4. Homburger, F., Tregier, A., *Endocrinology,* 1957, v61, 627.

5. Villee, C. A., *Ann. N. Y. Acad. Sci.,* 1959, v75, 524.

6. Homburger, F., Tregier, A., Grossman, M. S., *Endocrinology,* 1957, v61, 634.

7. Snell, G., *J. Nat. Cancer Inst.,* 1953, v13, 151.

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Comparison of Tolbutamide and Insulin Pre-Treatment Prior to Alloxan Induced Diabetes. (25267)

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Introduction of sulfonylurea compounds as oral hypoglycemic agents in therapy of mild diabetes has stimulated numerous studies to determine the mechanism of action of these drugs. Comprehensive symposia of these works have been published(1-4). Many investigators interpreted their findings as demonstrating that these drugs stimulate release of insulin by the pancreas and produce their hypoglycemic effect by increasing amount of available insulin. Insulin and glucose partially protect starved animals from alloxan diabetes(5) while starvation potentiates diabetogenic activity(6). This study was undertaken to compare the effect of insulin pre-treatment with tolbutamide pre-treatment on the diabetogenic potency of alloxan in rats.

Methods. Sprague-Dawley virgin female rats, weighing between 130-180 g, were used. In any one series the average weight of individual groups within that series did not vary by more than 5 g. The diabetogenic agent (alloxan-monohydrate) was injected subcutaneously on the basis of its anhydrous weight at a dose of either 150 or 175 mg/kg of body weight. Glucose was given either orally *ad lib* or injected intraperitoneally at a dose of 5 g/kg of body weight in the form of a 20% aqueous solution. Tolbutamide* was injected subcutaneously in all groups at a dose level of 200 mg/kg at a site on the opposite side of the body from that of the alloxan injection. Commercial crystalline zinc insulin was diluted in saline and injected subcutaneously at a dose of 1 unit/kg. A modified Somogyi-Nelson technic(7,8) was used for blood sugar determinations. Blood was taken from the tail of non-fasting animals from 24 hr through 6 days after administration of alloxan. Normal blood sugar range was found to be between 60 and 120 mg%, using the above technic. Microscopic sections were prepared of the pancreas, kidney and liver from animals in each group studied. All tissues were stained with hematoxylin-eosin (Harris). In addition the pancreatic tissues were stained according to Gomori for beta granules. For liver glycogen studies the animals were sacrificed by breaking the neck. Duplicate liver samples were rapidly removed, weighed and homogenized in 30% KOH. Glycogen determinations were done by the method of Hawk, Oser and Summerson(9). *Series I.* Following fasting period of 48 hr 15 animals were divided into 3 equal groups and additionally pre-treated as follows: 1) no further treatment; 2) glucose oral *ad lib* 6 hr; 3) tolbutamide 200 mg/kg subcutaneously followed by glucose oral *ad lib* 6 hr. The above pre-treatment was followed by subcutaneous injection of 175 mg/kg of alloxan. *Series II.* After 48 hr fasting period another series of animals were divided into 4 groups and further pre-treated as follows: 1) no additional treatment; 2) glucose oral *ad lib* 3 hr; 3) tolbutamide 200 mg/kg followed by glucose oral *ad lib* 3 hr; 4) insulin 1 unit/kg followed by glucose oral *ad lib* 3 hr. Alloxan at a dose level of 150 mg/kg was injected subcutaneously into all 4 groups following the above pre-treatment. *Series III.* Thirty animals were fasted 48 hr before intraperitoneal injection of 5 g/kg of glucose. These rats were then divided into 3 equal groups and treated

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