Experimentally Induced Benignancy of Neoplasm*†

V. The Influence of Hormones on the Host's Resistance to Implanted Neoplasm

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Several authors have reported the experimental production of neoplasms with estrogenic substances administered parenterally. These observations include not only the production of new growths in primary and secondary sex organs, but also of subcutaneous sarcoma by the implantation of crystalline estrone, as demonstrated by Gardner, Smith, Strong, and Allen (9).

Regarding the first group of observations, it is still debatable whether the hormone actually stimulates to malignancy, or merely hastens the development of tissue to the point where an independent predisposition to cancer may become effective. In the case of mammary tumor this point has been discussed by Lacassagne (15), by Geschickter (11), and by Nathanson and Andervont (18). In the case of the uterus, Gardner and Allen (10) classified seriatim the pathological stages which precede recognizable malignant tissue; *i.e.,* hyperplasia, secretory epithelial activity, stromal fibrosis, and pyometra. In this first group it is not clear that the action of the hormone is that of a true carcinogen.

In the second group, however, the female sex hormone appears to produce the same reaction as methylcholanthrene and other carcinogens, and these interesting demonstrations of carcinogenic effect might suggest at first sight that an excess of estrogenic hormone in the body always favors malignant growth. It is possible, however, to demonstrate another effect of the hormone, as reported from this laboratory in another publication (19).

This effect is not concerned with carcinogenesis, but rather with the fitness of the animal host as an animated culture medium for supporting the continued growth of a small island of tumor consisting of cells already clearly malignant. It has been demonstrated by Haddow and Robinson (12) and by Haddow, Scott, and Scott (13) that carcinogenic agents inhibit the growth of transplantable tumors and general body growth. These authors suspected from the work of Gardner *et al.* (9) that estrone might have a similar effect, but their experiments were negative. In our previous paper, however, it was brought out that such an effect could be demonstrated provided (a) the genetic constitution of the host were favorable, or (b) that the host's resistance against the neoplasm were increased by preliminary "immunization," either by live tumor cells, by non-viable tumor cells or even by tumor extract.[1]

Estrogens and immunity.—These experiments and the experiments reported in the present communication were performed with implanted sarcoma 180, in order to avoid confusion with the problem of carcinogenesis described above. The tumors were borne by 2 pure strains of mice; *i.e.,* black C57 and Bagg albino, strain A. It was found that in the black C57 strain some inhibition of a tumor growth did occur in mice treated with estrogens, as summarized in Table I. On the other hand, the albino animals showed no difference in the rate of growth when treated with estrone in huge doses. Nevertheless, when these albino animals were immunized first, the immunity was markedly enhanced, as shown in Table II (experiments 6a and 6b), which reveals a single but typical experiment. In brief, the control animals grew tumors in 98 per cent of cases, the immunized animals in 50 per cent of cases, and the hormone treated animals, following immunization, in no case.

When the immunity was not so efficient, some animals grew tumors as shown in Table II (experiments 7-9c), but even then the growth of these tumors was slow. Furthermore, these tumors behaved like benign

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[1] This "immunization" procedure is described elsewhere (20). Throughout this paper the term "immunity" is used in a specialized sense to indicate resistance against tumor growth on the part of the host.

tumors in many cases. For example, of 131 control animals, 89, or 68 per cent, were dead within a few weeks. On the other hand, after 5 months 76 per cent of 29 treated animals were living, and in some the tumors had regressed. This longevity of the treated animals emphasizes the effectiveness of the treatment.

TABLE I: INHIBITION OF TUMOR GROWTH BY ESTRONE

	Controls for virulence	Dose of estrone: 50 I.U. Group 1	Dose of estrone: 200 I.U. Group 2
Summary of experiments 1, 2, and 3 on {black males, C57; black females, C57}			
Number of animals	46	12	50
Mean tumor diameter at 3 weeks, mm.	16.5	11.0	11.8
		100 I.U.	
Summary of experiments 4, 5, and 6 on {albino males, strain A; albino females, strain A}			
Number of animals	61	57	
Mean tumor diameter at 3 weeks, mm.	13.4	12.4	
Mean tumor diameter at 4 weeks, mm.	17.0	17.5	

TABLE II: ENHANCEMENT OF IMMUNITY IN DIFFERENT DEGREES BY ESTRONE

	Controls: Virulence	Controls: Immunity	Estrone treatment: Hormone alone	Estrone treatment: Hormone after immunization
	Experiments 6a and 6b combined			
Number of animals	52	16	..	28
Mean tumor diameter at 3½ weeks, mm.	19	9.1	..	0
Number of takes	51 *	8	..	0
Percentage of takes	98 *	50	..	0
	Experiments 7, 8, 9a, 9b, and 9c combined			
Number of animals	51	52	53	70
Mean tumor diameter at 4 weeks, mm.	20.0	13.1	19.0	8.7
Number of takes	49 *	31	53	21
Percentage of takes	96 *	60	100	30

* A second inoculation showed that susceptibility was really 100 per cent. In other words, the failure to take was due to a lapse in technic.

The following fact indicates that the inhibition is primarily an effect on the host. When small, slowly growing tumors, borne by immunized hosts, are reimplanted into untreated mice, the new growths prove highly malignant.

EXPERIMENTAL METHODS

A detailed description of the methods employed and dosage used in these studies of 632 animals is presented in another report, together with a statistical analysis of results and a more extensive survey of the pertinent literature (19). It should be noted that the use of mean diameter as a means of tumor size tends to minimize differences in tumor growth. The data summarized above would be more striking if tumor volumes had been calculated from the 3 diameters, as $V = 4/3\ \pi\ abc$, or by the prolate-spheroid formula ($4/3\ \pi\ ab^2$), as used by Emge and Murphy (6). The smaller tumors, furthermore, showed a low rate of mitotic activity, as reported elsewhere (3). Both mitotic counts in colchicine-treated tumors and counts obtained by direct microscopic study showed a significant correlation between mitotic activity and tumor size.

In summary, these large doses of estrone are of interest, first, because they picture estrogen as a motivator for benignancy, and, second, because they concern the suppression of a tumor made up of nondescript fibrous tissue unrelated to sex functions. The mechanism whereby this suppression is produced remains obscure, but certain features of the problem have already been explored, as follows:

Pituitary and thyroid.—In the Program of the Third International Cancer Congress, Ball, Samuels, and Schott (1) have discussed the influence of the hypophysis on the growth of malignant tumors in rats. Likewise, Cramer and Horning (4) have pointed out that the pituitary reacts to the growth of tumors in animals and that the adrenal often is involved. The effect of estrone observed by us might be explained, therefore, on the basis of a rearrangement of the balance between various endocrine glands of the animal host. On the other hand, it might conceivably be related more directly to the sterol structure of estrone. In order to test these possibilities we have continued our observations by noting the effect of anterior pituitary growth hormone, of thyroxine and of castration. These results are summarized below in Tables III, IV, and V and are seen to be essentially negative. Similar studies previously reported by Meyer, McTiernan, and Aub (16), but without preliminary immunization, were also negative. These data supplement studies on the adrenal by Ball and Samuels (2), who also have thus far been unable to explain the connection between the growth of neoplasms and the endocrine glands.

Testosterone.—The second possibility; namely, a peculiarity related to sterol structure, has also been tested in part by the use of other sterols. The results with testosterone propionate are summarized in Table VI and are negative, even though the dosage used was 0.25 mgm. of the propionate every other day for 1 month. Figures 1 and 2 indicate the degree of scatter in 1 such experiment.

Other authors have reported observations with testosterone, some of them positive. Flaks and Ber (7, 8), for example, have described an anticarcinogenic action

in mice painted with methylcholanthrene. Similarly, Duran-Reynals (5) showed that an aqueous extract of the testicles of rats and rabbits inhibited the transplantability and growth of the Brown-Pearce epithelioma; and Mu (17) produced increased susceptibility of rabbits to Brown-Pearce epithelioma by administering estrogens, from human pregnancy urine, to male hosts. Heiman (14) obtained a like result with mammary fibro-adenoma in castrated rats injected with hormones. In short, the epithelial tumors showed precisely the reverse behavior to that of sarcoma 180 with regard to these sterols.

It is planned to test other sterols in order to explore further the effect of chemical structure in this respect. Investigations on progesterone are now in progress, but are not yet completed. Likewise, observations are being made with diethylstilbestrol, which is a powerful estrogen but not a sterol.

It should be emphasized that these data have nothing to do with carcinogenesis. Instead, they concern

TABLE III: EFFECT OF ANTERIOR PITUITARY GROWTH HORMONE ON THE GROWTH OF MALIGNANT TUMORS

	Controls		Growth hormone *	
	Virulence	Immunity	Hormone alone	Hormone after immunization
Number of animals	12	8	15	7
Mean tumor diameter, 3½ weeks	16.5 mm.	9.5 mm.	14.6 mm.	11.2 mm.
Number of takes	12	4	15	4
Per cent takes	100%	50%	100%	57%
Average weight at start	25.0 gm.	22.0 gm.	26.3 gm.	22.8 gm.
Average weight at finish	29.2 gm.	24.0 gm.	30.0 gm.	27.0 gm.

* The material used was a preparation of Growth Complex No. 470, of Ayerst, McKenna, and Harrison, prepared from the anterior lobe of the pituitary of the hog. It contained 100 rat units (Collip) of the growth hormone, 10 rat units (Collip) of adrenotropic hormone, and an indeterminate amount of prolactin. Dosage: 0.5 rat unit (Collip) every other day for 4 weeks.

TABLE IV: EFFECT OF THYROXINE ON THE GROWTH OF MALIGNANT TUMORS

	Controls		Thyroxine *	
	Virulence	Immunity	Hormone alone	Hormone after immunization
Exp. 1				
Number of animals	31	30	.....	18
Mean tumor diameter, 3½ weeks	14.2 mm.	6.2 mm.	.....	5.3 mm.
Number of takes	31	18	.....	8
Per cent of takes	100%	60%	.....	45%
Exp. 2				
Number of animals	6	8	9	8
Mean tumor diameter, 2 weeks	11.7 mm.	7.2 mm.	10.3 mm.	9.8 mm.
Number of takes	6	6	9	6
Per cent of takes	100%	75%	100%	75%
Exp. 3				
Number of animals	5	9	15	20
Mean tumor diameter, 4 weeks	13.0 mm.	7.0 mm.	13.1 mm.	3.6 mm.
Number of takes	5	2	15	5
Per cent of takes	100%	22%	100%	25%

* Squibb's crystalline racemic thyroxine. Dosage: 0.005 mgm. thyroxine dissolved in alkali, by intramuscular injection every other day for 4 weeks.

TABLE V: EFFECT OF CASTRATION ON THE GROWTH OF MALIGNANT TUMORS

	Experiment	Controls C57		Castrated mice C57	
		Males	Females	Males	Females
Number of animals	1	6	6	12	12
Mean tumor diameter at 3 weeks, mm.	..	18.0	17.0	19.5	22.0
Mean tumor diameter at 4 weeks, mm.	..	21.0	19.0	25.0	27.0
Average deviation at 4 weeks, mm.	..	± 2.7	± 1.0	± 3.3	± 1.2
Number of animals	2	15	15	15	15
Mean tumor diameter at 3 weeks, mm.	..	20.1	14.0	18.5	18.0
Mean tumor diameter at 4 weeks, mm.	..	22.0	19.8	21.8	23.5
Average deviation at 4 weeks, mm.	..	± 1.7	± 3.8	± 2.2	± 2.8

the possibility of making the organism an unfit medium for the continued growth of malignant cells. The results thus far obtained do not distinguish between a strictly endocrinological effect and an inhibitory effect like that observed by Haddow (13). Even though the mechanism remains obscure, the results suggest that the fate of an implanted tumor can be determined in part by the endocrinological status of the host.

Summary and Conclusions

Several authors have reported the production of neoplasms with estrogen, administered parenterally. These interesting demonstrations of carcinogenic effect might suggest that an excess of estrogenic hormone in the animal body always favors malignant growth. It is possible, however, to show that estrone may inhibit the growth of implanted neoplasm. In this case, the sarcoma behaves as if benign in several characteristic

Table VI: Effect of Testosterone on the Growth of Malignant Tumors †

	Controls		Testosterone	
	Virulence	Immunity	Hormone alone	Tail treatment plus hormone
Exp. 1—Males				
Number of animals	8	11	14	15
Mean tumor diameter	15.8 mm.	11.5 mm.	16.9 mm.	11.0 mm.
Number of takes	8	7	14	4
Per cent of takes	100%	64%	100%	27%
Exp. 2—Males				
Number of animals	16	22	16	23
Mean tumor diameter	18.0 mm.	8.4 mm.	17.3 mm.	10.6 mm.
Number of takes	16	13	16	14
Per cent of takes	100%	59%	100%	61%
Exp. 1—Females				
Number of animals	12	20	10	17
Mean tumor diameter	11.8 mm.	5.2 mm.	13.2 mm.	8.5 mm.
Number of takes	10	6	10	3
Per cent of takes	84% *	30%	100%	17%
Exp. 2—Females				
Number of animals	17	23	16	23
Mean tumor diameter	17.7 mm.	10.1 mm.	16.1 mm.	6.8 mm.
Number of takes	17	7	16	7
Per cent of takes	100%	30%	100%	30%

† Dosage: 0.25 mgm. of testosterone propionate in oil, by intramuscular injection every other day for 4 weeks.
* Two reinoculations were successful, thus bringing the true percentage of takes to 100 per cent.

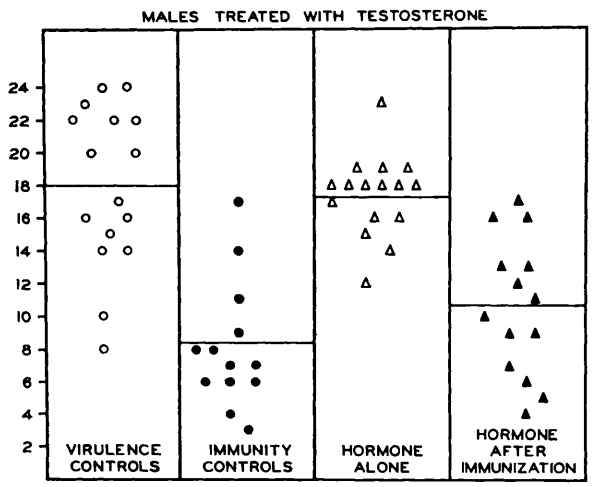

Fig. 1.—Testosterone in large doses failed to enhance the resistance of male mice against growth of sarcoma 180.

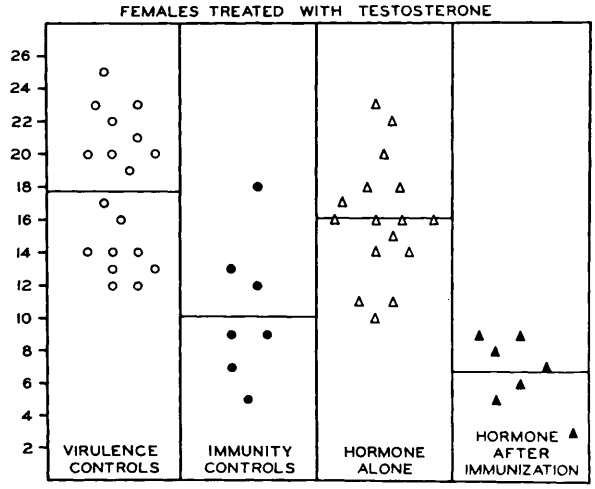

Fig. 2.—Testosterone in large doses failed to enhance the resistance of female mice against growth of sarcoma 180.

respects. This inhibition is probably not a direct inhibition of tumor tissue, but rather the enhancement of a primary resistance mechanism.

Homologous experiments performed with androgen, anterior pituitary growth hormone, and thyroxine were negative. Castration of females may decrease slightly the resistance to tumor growth, but the effect is slight at best.

These data have nothing to do with carcinogenesis, but rather concern the possibility of making the organism an unfit medium for the continued growth of malignant cells. They suggest that the fate of an implanted tumor is partly determined by the endocrinological status of the host.

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