

GENETIC AND ENDOCRINE STUDIES ON A TRANSPLANTABLE CARCINOMA OF THE OVARY

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INTRODUCTION

A CARCINOMA of the ovary has been maintained by successive transplantations in mice of the CBA strain for nearly three years. In the first study with this tumor, it was determined that only normal males of the CBA strain would show progressively growing tumors during a three-month period. It was also ascertained that the growing transplanted tumor secreted appreciable amounts of estrogen. The criteria of hormone secretion of the transplanted tumors were: (a) long continued estrous vaginal smears which, after removal of the tumors reverted to the diestrous type in castrate females; and (b) growth of the rudimentary mammary glands of males as the transplanted tumors grew. Insufficient hormone was secreted to affect the pelves (STRONG, GARDNER and HILL 1937).

The above conclusions reopened the problem of immunity to this transplanted neoplasm. The transplantation of neoplastic tissue (primarily based upon carcinoma of the mammary gland) demonstrated that the progressive growth of the tumor was determined by genetic factors inherited according to the accepted laws of mendelian heredity. No functional characteristic of the grafted tumors, however, has been demonstrated in these tumors growing in genetically controlled strains of mice. The presence of multiple factors underlying susceptibility and immunity to the previously grafted tumors has complicated the physiological factors that should have been evident. In the only case in which susceptibility and immunity to a grafted tumor was apparently controlled by a single mendelian factor, the especially selected strain of mice was lost before adequate physiological proof was obtained (STRONG 1926).

The present tumor, a carcinoma of the ovary, was a suitable tissue for our purpose. The sex difference encountered in the natural susceptibility or immunity to the tumor in inbred CBA mice suggested that a simple genetic mechanism was involved. Again, the hormone factors involved in sex physiology are so well known that ample opportunity of investigating

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this problem in relation to the grafted tumor should not be further delayed. The secretion of estrogen by the tumor during its growth as a transplant is a type of physiology which can be easily measured. Other hormone preparations known to influence (1) follicular development of the ovary (the tumor probably arose from granulosa cells) and (2) sex physiology, should therefore be investigated. Finally the action or interplay of genetic and hormonal agencies in building up the physiological behavior of the individual seems to be indicated.

RESULTS

The genetic factors involved in the successful growth of the grafted tumor were studied by (1) the inoculation into normal male and female mice of the CBA (STRONG 1936) strain, (2) males and females of the A strain (STRONG 1936a) and (3) the hybrids of the first two filial generations produced by crossing individuals of these two inbred strains.

Inoculation into normal CBA mice

One hundred and sixty-eight female and 153 male mice of this strain were inoculated with the tumor. All males grew the tissue progressively (100 percent susceptible). Only eight of the normal females grew the tissue,

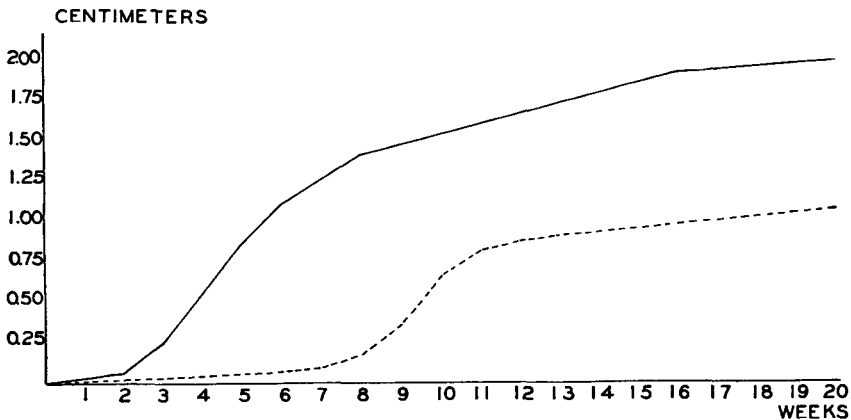


FIGURE 1.—The average growth rate of the transplant in (a) male mice (solid line) and (b) female mice (dash line) of the CBA strain. Time in weeks is plotted along the base line; size of tumor (greatest diameter in centimeters) is plotted along the vertical line.

and of these eight, seven occurred in the first two transfer generations. After a lapse of time (approximately a year) a new series of mice of the CBA strain were inoculated with the tumor and the females were kept for a considerable period after the males had all died with large tumors. Approximately eighteen percent of normal females of the new series eventually grew the tissue. In another experiment, a few female mice grew the

transplanted tissue to the size of a pea over a period of eighteen months. The sexual difference encountered was not an absolute one but rather one of degree. Whether more normal females of the CBA would later grow the tissue is of course not shown by the present data. The comparative analysis of susceptibility and resistance to the grafted tumor was significant only when the time relationships existing after the mice were actually inoculated were taken into consideration. The transplants grew much faster in males than in females (fig. 1). The tumors after a period of relatively rapid growth, were infiltrated with heavy calcified deposits, thus becoming rock-like, after which they did not increase much in size.

Results of inoculation into F_1 individuals

Since a sex difference was encountered in the inoculation of normal individuals of the CBA strain, the outcross to the A strain was made in both directions. Seventy-nine mice produced by crossing CBA females

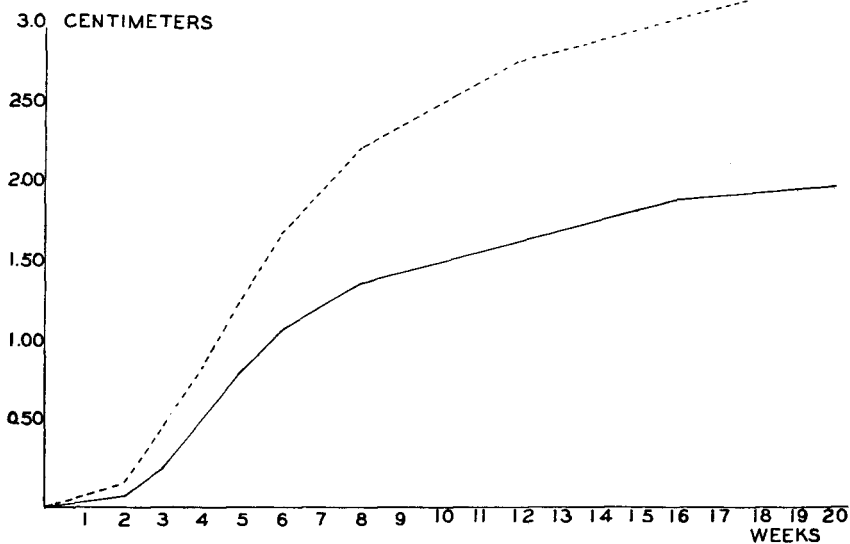


FIGURE 2.—The average growth rate of the tumor in (a) normal male mice of the CBA strain (solid line) and (b) F_1 male mice obtained by a cross between the CBA and A strains. Heterosis increases the growth rate of the grafted tumor.

with A males were inoculated with the tumor; of these, 26 were males and 53 females. All of the 26 males grew the tissue progressively; whereas only 10 of the 53 females grew the tumor. The percentage of female F_1 individuals growing the tissue (18.8%) was approximately the same as that obtained in purebred CBA females. In the other cross when A females were mated to CBA males, 91 mice were obtained. Of these, 44 were males and

47 females. All the males and 9 of the females grew the tumor. Of the F_1 females produced by this cross 21.3 percent thus grew the tumor or approximately the same proportion as that obtained in the first F_1 population and in the original CBA females. Apparently neither sex linkage nor sex limited inheritance was involved in susceptibility to this transplant. That both the males and females of the F_1 generation give the same percentage of susceptible individuals would indicate, however, that susceptibility to the transplanted tissue is intrinsically determined. The evidence indicates that a non-genic sex-limited influence is probably involved.

Male mice of the F_1 generation grew their tumors at a more rapid rate than male mice of the CBA strain, as indicated in figure 2. This finding has been encountered several times and indicates that heterosis is involved in the rate at which a transplanted tumor grows, as postulated by STRONG (1926a).

Results obtained in the F_2 generation

The data obtained by inoculating the tumor into F_2 individuals are presented in table 1.

TABLE 1

	CBA ♀ × A ♂			A ♀ × CBA ♂			AVERAGE PERCENT
	+	-	PERCENT	+	-	PERCENT	
	+			+			
♀	8	148	5.1	16	196	7.5	6.5
♂	20	80	20.0	40	52	38.4	29.4

The data obtained in the male mice will be discussed first. It appears that multiple mendelian factors (probably four) are involved in the successful growth of this transplantable carcinoma of the ovary. The expectation for independent multiple factors in the F_2 generation is given in table 2.

TABLE 2

NO FACTORS	RATIO	PERCENT
+		
1	3:1	75.00
2	9:7	56.25
3	27:37	42.18
4	81:175	31.64
5	243:781	23.73

The percentage obtained (29.4) with this F_2 generation of male mice lies between that expected for four (31.64%+) or five (23.74%+) factors, nearer that expected for four factors.

The genetic analysis of susceptibility to the transplant for female mice of the same inbred strain was, however, not so clear. Definite ratios were

obtained both in pure strain individuals as well as in the outcross generations to pure strains thus indicating that intrinsic factors are involved in the process of transplantation. The same diminution of susceptibility is obtained in the F₂ individual females as was seen in a similar analysis for males in the same experiment. These comparative data are shown in table 3.

TABLE 3
PERCENT +

	♂	♀
CBA	100	18.2
F ₁	100	20.1
F ₂	29.4	6.5
F ₁ >F ₂	3.4	3.1

It is tentatively concluded, from the above analysis, that approximately four mendelian factors are involved in the successful transplantation of the carcinoma of the ovary in both males and females. The complete multiple-factor complex manifests itself in male mice only. Apparently some physiological mechanism within the female inhibits or prevents the full manifestation of its genetic constitution.

In order to test out this hypothetical mechanism that underlies susceptibility and resistance to the transplant an endocrinological investigation was undertaken.

STUDIES OF HORMONE TREATMENT ON TUMOR RESISTANCE

Effect of castration. Thirty-one male mice of the CBA strain, castrated at approximately six weeks of life, were inoculated with the tumor two weeks later. Of these, only fourteen grew the transplant. When the growth

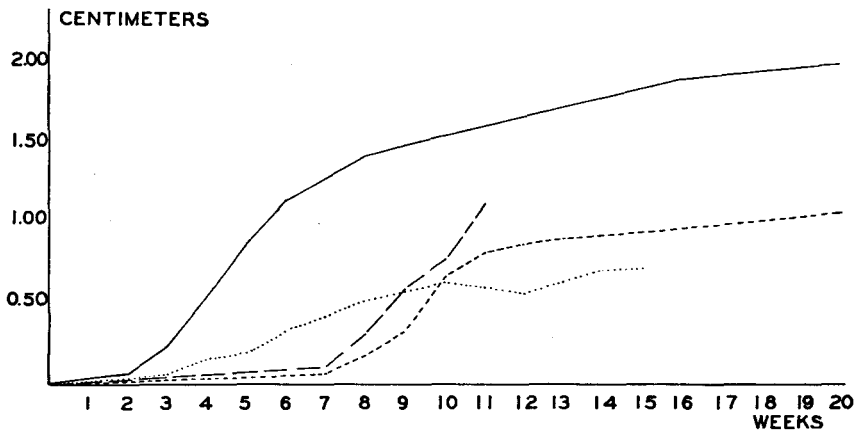


FIGURE 3.—The average growth rate of the transplant in (a) normal male mice (solid line), (b) normal female mice (long dash line), (c) castrate male mice (dotted line) and (d) male mice receiving estrogen (short dash line).

rate of the tumor is compared to that obtained in normal male and female mice of the same strain (fig. 3) it is seen that castration not only diminished incidence but also decreased the growth rate of the transplant similar to that obtained in normal female mice of the same strain.

Twelve female mice of the CBA strain were castrated at six weeks of age and inoculated with the transplant. Four of these grew the tissue progressively and slightly faster than did normal female mice of the same strain. Daily vaginal smears disclosed the presence of some estrogen but in insufficient amounts to keep the castrate female in continuous estrous.

Hormone injections. Since the incidence and growth rate of this transplanted ovarian carcinoma was related to the presence of the gonads, especially the testes, it might be affected by the sex hormones or gonadotropic hormones. This hypothesis was tested by injection of various hormones into 115 CBA mice in which the tumor had been transplanted under the skin in the region of the right axilla. Sixty females and 55 males were used, half of the females and 25 of the males were castrated. Five castrate and five non-castrate mice of each sex served as controls for the similar group which received daily injections respectively, of 25 i.u. of estrogen-2, (Folliculin Benzoate or Progynon B₂),² $\frac{1}{4}$ mg. Progestin (Prolutin and Progestin $\frac{1}{2}$ r.u. follicle stimulating hormone (Prephysin), 2.5 r.u. pregnant mare's serum (or Gonadin serum) and 0.05 cc. of egg albumen. The 0.05 cc. egg albumen was used as a control for the protein in the gonadotropic hormones. As Progestin had only a slight inhibitory effect on the growth rate of the tumor, it was used only in the normal male and female series, and 200 gamma (weekly) of male hormone (testosterone propionate-9) was used in the castrates.

RESULTS

The growth rate of the transplants in male mice receiving pregnant mare's serum was not affected (fig. 4). On the contrary, female mice receiving pregnant mare's serum grew their transplants at a more rapid rate than their controls (fig. 5). This enhanced growth rate may be due to (1) a "mutation" of the tumor similar to those encountered by STRONG

² 1. The Folliculin Benzoate was obtained from the British Drug House, London, England, and Progynon B was supplied through the courtesy of Dr. E. Schwenk of Scherring Corporation.

2. The Prolutein used was obtained through the courtesy of Dr. E. Schwenk of Scherring Corporation, and the Progestin through the courtesy of Dr. O. Kamm of the Parke-Davis Company.

3. The follicle stimulating hormone, Prephysin, was obtained from Chappel Bros., Inc., Rockford, Illinois.

4. The pregnant mare's serum was supplied by the Parke-Davis Company, through the courtesy of Dr. O. Kamm and the Gonadin was purchased from Cutter Laboratories, Berkeley, California.

5. Testosterone Propionate, "oreton," was supplied through the courtesy of Dr. E. Schwenk of the Scherring Corporation.

(1924, 1926b), BITTNER (1930, 1931) and CLOUDMAN (1932) with transplantable carcinomata of the mammary gland, or (2) the stimulation of the growth rate indirectly or directly without having undergone any cellular

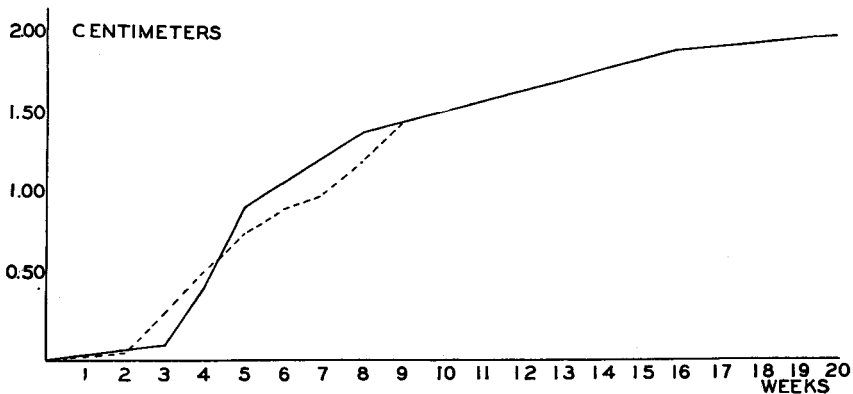


FIGURE 4.—The average growth rate of the tumor in (a) male mice (solid line) and (b) male mice receiving pregnant mare's serum (dash line). There is no difference between the two curves.

change. In order to test these two hypotheses, subsequent grafts were made from these enhanced growing tumors. The growth rate of these was the same as that obtained with the original tumor in male mice of the CBA strain. The second conclusion, therefore, seems to be the more logical one. In addition to a very rapidly growing tumor, one of the mice receiving pregnant mare serum also had several metastatic nodules in the lungs. This

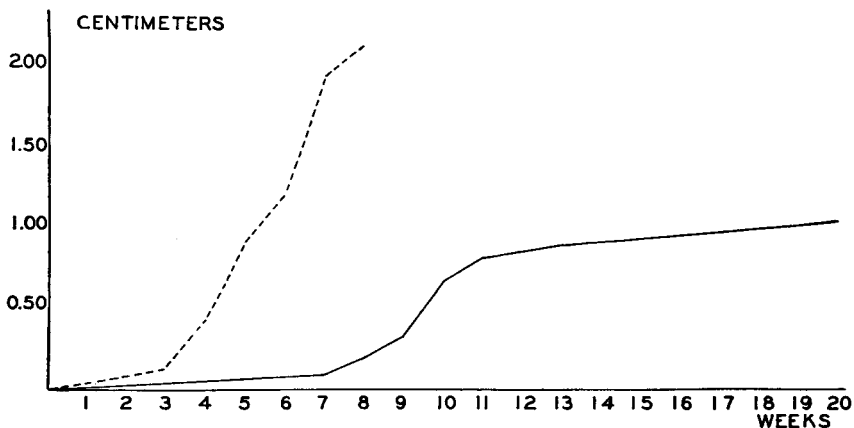


FIGURE 5.—The average growth rate of the transplant in (a) female mice (solid line) and (b) female mice receiving mare's serum (dash line). Female mice receiving pregnant mare's serum grow their tumors at an enhanced rate.

is the only mouse of several hundred examined that had a metastasis from this tumor. The tissue from the lung metastasis has been carried on by subsequent transplantations for four generations without any obvious

deviation from the growth rate of the original transplanted tumor, or evidence of further metastatic activity.

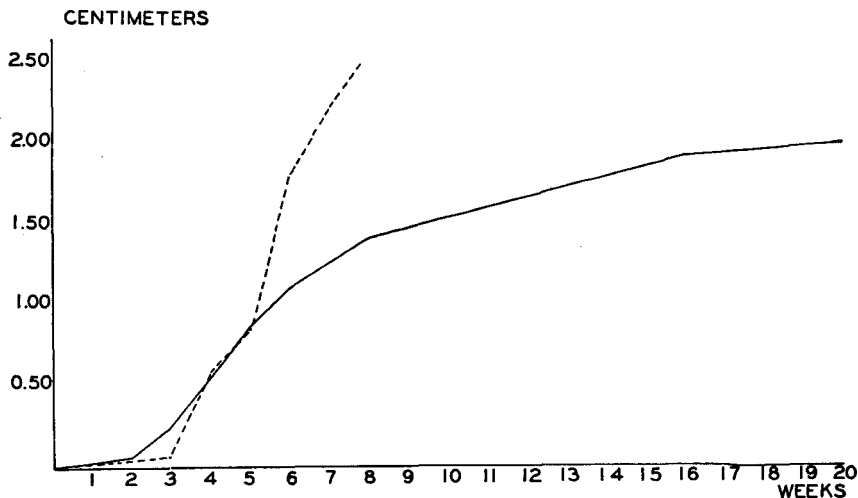


FIGURE 6.—The average growth rate of the transplant in (a) male mice (solid line) and (b) male mice receiving follicle-stimulating hormone (dash line).

The injection of the follicle-stimulating hormone had no detectible effect on the growth rate of the tumor or the survival time of normal female mice. On the other hand, it had a significant stimulating effect on the transplant growth in normal males (fig. 6).

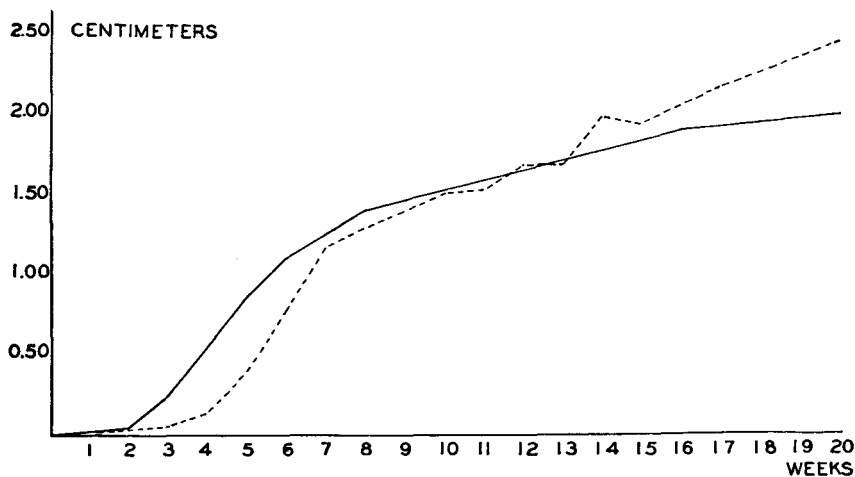


FIGURE 7.—The average growth rate of the tumor in (a) male mice (solid line) and (b) male mice receiving prolutin (dash line).

Prolutin may have had a slight inhibitory action on the growth of the carcinoma of the ovary, since the growth curve lagged behind that ob-

tained for the controls—and eventually approached the normal curve after the injections of prolutin were discontinued (fig. 7).

The injection of estrogen into both males and females inhibited the growth rate of the transplanted tumors (figs. 3 and 8).

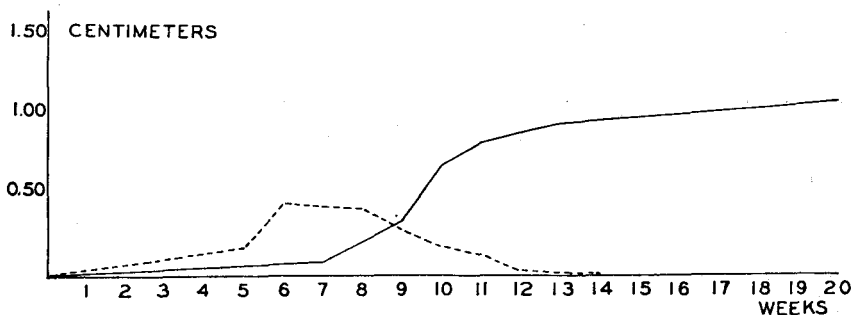


FIGURE 8.—The average growth rate of the transplant in (a) female mice (solid line) and (b) female mice receiving estrogen (dash line). Female mice receiving estrogen completely regressed their tumors.

The growth rate of the implanted tumor was not altered by the injection of any of the hormone preparations when the individual (male or female) had been previously castrated. The injection of egg albumen did not affect the growth rate of the tumor. The comparative effect obtained by the various injections is given in table 4.

TABLE 4
FEMALE

	FEMALE		MALE	
	NORMAL	CASTRATE	NORMAL	CASTRATE
Mare's Serum	+	o	o	o
F.S.H.	o	o	+	o
B.D.H.	-	o	-	o
Prolutin			-	
Egg Albumen	o	o	o	o
Male Hormone				o

GENERAL DISCUSSION

Though this tumor arose in the ovary a delayed or complete failure of its development following transplantation into intact female mice soon became apparent. Whether the tumor arose under altered hormonal conditions or underwent changes following transplantation afforded two possibilities for speculation.

Since the genetic study indicated a non-genic sex-limited regulation of transplantation and rate of growth the possibility of hormonal factors was investigated. The intact male afforded the optimum environment for the transplant; the intact female the least satisfactory host. Orchidectomy inhibited the growth of the tumor.

The increased rate of growth of the transplanted tumors in normal females receiving pregnant mare's serum might indicate that this effect was obtained by the action of the ovary. F.S.H. was, however, without effect. The failure of estrogen or progestin injections were not compatible with such interpretation. The lack of effect of pregnant mare's serum in intact males indicates an absence of any direct effect of this gonadotropic principle. The present experiments fail to explain definitely the role of endocrines indicated by the differences of growth of the transplant in the males and females. Some tumors particularly certain adenomas of genital tissues respond to their hormonal environment. The more malignant tumors have failed to respond to most hormonal deficiencies, presence or excesses, unless the general condition of the animal is greatly affected. It is particularly interesting that this comparatively malignant tumor has also retained to some extent the physiological activity of the tissue of origin.

The tendency of the tumor to calcify, particularly when growing slowly, in intact females also merits further investigation.

The genetic theory of transplantation of neoplastic tissue as postulated by STRONG formulated the conception that the fate of neoplastic tissue within the body was controlled by a reaction between the tumor and the host. The reactivity of the host is controlled by its intrinsic genetic constitution; that of the tumor is also determined by an internal genetic constitution. The data of STRONG, BITTNER and CLOUDMAN have clearly demonstrated that the genetic constitution of the tumor may change from time to time presumably by a process similar to somatic mutation.

The present data, on the other hand, demonstrate that the reactivity of the host toward a transplantable carcinoma of the ovary may be influenced by the injection of hormone solutions or by gonadectomy.

The genetic data demonstrate that susceptibility and resistance to the transplant are determined intrinsically by multiple mendelian factors. There are at least two hypotheses for the explanation of the relationship between genic and hormone action. The first one maintains that the early development of the individual is determined by genic action. This is eventually replaced by hormone action during the adult physiological life of the individual. The second hypothesis maintains that genic action and hormone action are both present simultaneously. With the accumulated data on the effect of genes throughout the entire life span of the individual and on the inheritance of disturbances of the endocrine system itself, it seems more likely that hormone action may very well be controlled by or be concomitant with genic action.

It is more than probable therefore, that in this investigation the influence of hormone preparations on susceptibility and resistance to a transplantable carcinoma of the ovary has been demonstrated by the possibility

that the defect or variation received by the individual through its germ plasm has been made good by hormone therapy.

SUMMARY

The growth of a transplanted carcinoma of the ovary depends on the simultaneous presence of multiple mendelian factors (probably four). The growth rate of the tumor can be influenced, however, by internal secretions of the host. However, the endocrine factors have not been clearly worked out because of (1) the extreme calcification undergone by the tumor and (2) the extremely slow growth of the transplant in intact females.

The problem of tumor susceptibility and resistance is discussed; the interest inheres primarily in the fact that the tumor has retained the physiological activity of the tissue from which it originated (that is, the ovary).

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