Relation of Mammary Tumors to Mammotropes.* III. Hormone Responsiveness of Transplanted Mammary Tumors. (25623)

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The first paper of this series(1) reported induction of mammary carcinoma (MT) in rats by 3-methylcholanthrene (3-MCA). The second paper(2) described responsiveness of primary tumors to hormones. Both papers indicate a major role of the mammotrope (Mt) in induction and growth of mammary tumors (MT) of the rat. A subcarcinogenic dose of 3-MCA caused MT only in rats whose mammary gland was stimulated by mammotropic hormone (MtH), provided by grafts of mammotropic tumors (MtT). Ovariectomy and hypophysectomy retarded many and completely arrested some primary MT. Grafts of functional MtT (*i.e.*, MtH) caused resumption of tumor growth. The purpose of this study was (a) to determine whether primary grafted MT would respond to hormonal influences in the same way as original tumors on their natural hosts, (b) to develop responsive and autonomous MT of diverse types for screening procedures and chemicals of possible therapeutic value and (c) to analyze further the role of Mt in maintaining growth of MT.†

Methods. The grafts were made either by s.c. injection of tumor mince, or by placing tumor fragments in s.c. pockets. Large tumor fragments yield tumors faster than injection of tumor mince, but the latter is a more convenient procedure when large numbers of animals are needed. In each group 6 to 10 rats, 50 to 60 days old, were used. Thirty-three induced tumors were biopsied. Parts of the tumors were sectioned and studied histologically, parts grafted on variously conditioned and normal rats of the strain of origin.

Results. Table I summarizes results of 31 transplantation experiments of 22 strains of 3-MCA induced tumors in $F/_{Fu}$ rats. All tumors were autonomous in that they grew in normal males as well as females, but, with rare exceptions, all exhibited some degree of hormonal responsiveness as indicated by slower growth rates in males than in females. Table I indicates a marked decrease in latency periods in the course of subpassages in both females and males. However, summating data obscures the marked individual differences in growth rates of tumors in males and females, the magnitude of which is a good lead on degree of estrogen responsiveness of a given mammary tumor. Some MT strains were highly estrogen responsive, others were entirely estrogen independent. Most of them were in between (Table II). Loss in degree of responsiveness and increase in growth rate are characteristic features of most, if not all MT of this series (Table I,II).

Table III compares first and second passage of 3-MCA induced MT in $W/_{Fu}$ rats. Acceptance of the graft by all 405 $W/_{Fu}$ females (Tables III, IV) indicates a practical homozygocity of this strain, while failure of 7 males to accept similar grafts indicates occasional marked hormonal (estrogen) responsiveness. This vanished, however, on the next passage.

Half of the $W/_{Fu}$ tumors were fully autonomous in the original grafts (*i.e.*, there was no difference in latency period between males and females); half were hormone responsive, as indicated either by failure to take in males or by longer latency periods and smaller size of tumors in males.

A smaller number of MT arising in irradiated $W/_{Fu}$ rats (Table IV) and of spontaneous MT of both rat strains (Table V) were also studied. While 3-MCA induced tumors were almost all carcinomas, most of those induced by radiation were fibroadenomas(3).

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† For tumor and host strains, procedures and key references see preceding papers(1,2). Adrenalectomized rats were maintained on 1% NaCl in drinking water. The grafted MT strains were named after the number of animal in which tumors arose.

TABLE I. Comparison of First and Subsequent Passages of Mammary Tumors in W/Fu Rats, Induced by 3-MCA.

	Females				Males			
		No. of rats				No. of rats		
Passage	No. of exp.	Grafted	Tumor	Tumor latency*	No. of exp.	Grafted	Tumor	Tumor latency*
1st	21	193	191	(25-108) 51	11	66	64	(35-172) 97
2nd	7	137	137	(15-59) 32	8	65	63	(22-87) 52
3rd	2	66	66					
4th	1	40	40					
Total	31	436	434		19	131	127	

* days

TABLE II. Degree of Responsiveness of Grafted Tumors.

Degree of response*	1st passage No. of strains	2nd passage No. of strains
None	3	3
Slight	2	1
Moderate	2	1
Marked	3	1

* Rating was as follows: "Unresponsive" tumors were rated those which had about the same latency periods in males as in females; "slightly responsive" those tumors in which latency period in males was longer by not more than 50%; "moderately responsive" those in which this period in males was more than double; and "highly responsive" those with latency periods in males more than triple that in females.

Of 6 radiation induced tumors selected for serial transplantation, 3 were carcinomas and 3 were fibroadenomas (Table IV). All carcinoma strains of the W/Fu rats were fully autonomous (grew equally in males and females); of the 2 fibroadenomas studied, one was fully autonomous and the other grew slightly better in females. Considered as a group, W/Fu MT strains were less hormone responsive and had shorter latency periods than F/Fu strains. In one W/Fu strain latency period of the primary graft was 15 days, that of the first passage 6 days, and of the second passage 4 days. Latency period of another strain was 30 days in first passage and 18 days in second passage.

Hormonal influence. Maximal inhibition of MT in F/Fu rats was obtained by hypophysectomy, somewhat less by ovariectomy, and almost none by adrenalectomy (Table VI). Excellent stimulation of MT growth was obtained by MtT grafts (MtH).

MtH (MtT) can even overcome the extreme inhibition in males, which is probably due to androgens (Fig. 2). In experiments shown in Fig. 1, ovariectomy and hypophysectomy were performed on groups of 6 rats when the grafted MT measured 7 to 10 mm. Six matched tumor-bearing rats were left as

TABLE IV. Summary of Transplantation Data of Mammary Tumors Occurring in X-radiated W/Fu Rats.†

		Females		Males	
Type	Passage	No. of strains	No. rats grafted*	No. of strains	No. rats grafted*
Ca	1	3	110	2	16
	2	2	31	1	8
	3	1	8		
Fibroad.	1	3	50	2	13
Total		9	199	5	37

* All grew. † Obtained from Dr. C. J. Shellabarger.

TABLE III. Comparison of First and Second Passage of Mammary Tumors of 3-MCA Treated W/Fu Rats.

	Females			Males			
Passage	No. of strains	No. rats grafted*	Latency, days	No. of strains	No. rats grafted	No. +	Latency, days
1	9†	206	(21-84) 40	6	47	40	(21-253) 94
2	3	54		2	18	18	

* All grew. † 8 carcinoma and 1 fibroadenoma strains.

TABLE V. Transplantation of Spontaneous Mammary Tumors.

Rat strain	Tumor type	Passage	Females				Males		
			No. of passage	No. inj.	No. +	Latency, days	No. of passage	No. inj.*	Latency, days
F8	Fibroad.	Primary	2	38	38	13			
W1	"	"	1	10	10	60	1	15	60
		1st subp.	1	35	35	38			
W7	Ca	Primary	1	17	15	230	1	7	230
		1st subp.	1	20	20	30	1	5	30
W8	"	Primary	1	18	18	56			
		1st subp.	1	12	12	95	1	12	95

* All grew.

controls. The points in Fig. 1 represent averages. Note sharp resumption of tumor growth, following inhibition by operative procedures, upon subsequent MtT grafts.

In experiments shown in Fig. 2, grafted MT grew exceedingly slowly in 10 normal male rats. 56 days after MT graft, when the tumors were barely palpable, MtT was grafted on 5 MT bearing rats. MT growth was markedly accelerated by MtT (MtH) but remained slow without it.

In one experiment (Fig. 3), 20 rats bearing MT were divided in 2 groups when tumors measured about 7 mm; 10 rats were grafted with MtT, 10 remained as controls. Hypophysectomy was performed on all 20 rats 19 days later. MT on 10 rats bearing MtT grew progressively, while in those without MtT, shrank progressively. MtT (MtH) was necessary to maintain progressive growth of these MT in absence of the pituitary. However, the figures in Table VI are somewhat deceptive. The stimulating effect of MtH on *all* tumors listed in Table VI is due to the fact that only those tumor strains which responded to ovariectomy or hypophysectomy were challenged by MtT grafts.

TABLE VI. Hormonal Influences in Grafted Mammary Carcinomas in $F/_{Fu}$ Rats.

	Inhibition				Stimul. by MtH
	Ovex	Hypex	Adrex	Androg.*	
No. of strains	8	9	4	10	7
No. of rats	47	78	18	88	46
No. of exp.	9	12	4	14	8
Effects:					
None	1	1	3	5(4) †	0
Slight	3	3	1	1(3)	0
Moderate	1	2	0	4(3)	1
Marked	4	6	0	4(4)	7

* Comparative growth of tumors in normal males vs females.

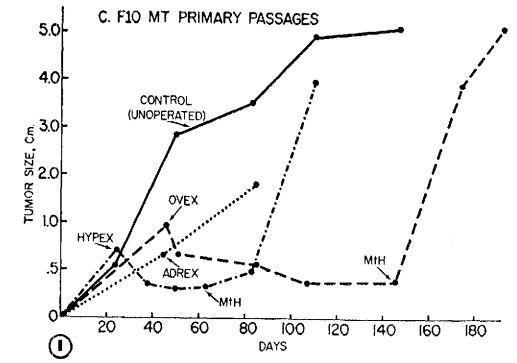
† First figure indicates prolongation of latency period, figure in parenthesis inhibition of tumor growth (size) in males *vs* females.

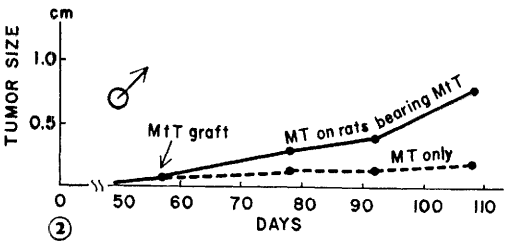
Differences in latency periods between males and females are probably due to differences in estrogen levels. Ovariectomy is more effective than would be expected on the basis of growth inhibition in males. This can be well explained by differences in estrogen levels.

There was no close relation between latency periods and hormone responsiveness; *e.g.*, F7 (Table VII), a non-responsive strain, had a latency of 27 days; 3 highly responsive strains (F5, F9, F10) had about the same latency periods (25-35 days).

Table VII attempts to answer the question whether hormone responsiveness of grafted MT's is similar to that of original tumors from which they were derived. Response to ovariectomy of original MT and first generation grafts was made in 3 cases, response to hypophysectomy in 5. There was excellent conformity between behavior of the original tumors and first generation grafts in 5 cases; in 2 there was a slight drop, and in 1 a slight gain in hormone responsiveness. On second passage, 2 tumors still remained highly responsive and 2 gained markedly in autonomy. Response to ovariectomy was compared to that of hypophysectomy in 6 first generation grafts, and in one 2nd generation graft. There was good conformity in all.

Table VIII summarizes the degree of hormonal influences on all grafted MT studied. The greatest inhibition was obtained by hypophysectomy; the least by adrenalectomy. The specificity of slight inhibition by the latter





MtT

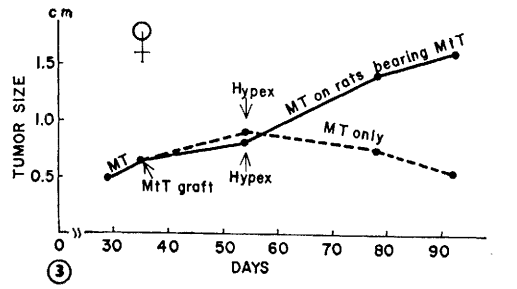


FIG. 1. Effect of ovariectomy and hypophysectomy on grafts of 3-MCA induced mammary carcinoma (Strain F10) in female rats. Each point represents avg of 6-10 rats. Note regression of tumors following surgery and resumption of growth following grafts of a MtT (supplying MtH).

FIG. 2. Exceedingly slow growth of mammary carcinoma (Strain F10) in 20 normal males (the point representing avg values) and marked stimulation of their growth in 10 of these rats following grafts of MtT.

FIG. 3. Maintenance of progressive growth of MT in hypophysectomized rats that had received MtT graft 19 days earlier and shrinkage of MT in those without it. The points are avg values on 10 rats.

(with one exception) is altogether doubtful.

Comments. Use of large numbers of rats in this transplantation series was necessitated by our aim to find stable estrogen-responsive tumor strains with varying grades of responsiveness, suitable for screening potential tumor inhibitors. This objective was not fully attained but the experiments point to technical difficulties which could be corrected. Histological examinations of mammary glands indicate that numerous cells of 3-MCA treated rats undergo neoplastic transformation throughout the glands. When grafts are made, it is likely that the more rapidly growing and independent clone outgrows the more slowly growing clone. This situation calls for clonal isolation of tumors, less intense carcinogenic treatment, and transplantation as soon as a tumor appears. There is no way known to counteract progression of a tumor, but repeated clonation may enable isolation of a desired clone. Preservation of wanted clones in the frozen state may enable their maintenance over periods of many years.

The 100% takes in numerous experiments involving 405 $W/_{Fu}$ rats of both sexes, as well as numerous other transplantation experiments, including leukemias and skin grafts (Maddock, personal communication), indicate

TABLE VII. Comparison of Responsiveness of Original *vs* Grafted Tumor.

Strain		Response* to Ovex	Hypex
C.F1	Orig.	+	+
	Graft I	+	
F2	Orig.		+++
	Graft I	++	++
F3	Orig.		+++
	Graft I	+++	
	" II		+
F5	Orig.		+++
	Graft I		+++
	" II	+++	+++
F7	Orig.		0
	Graft I	0	0
F9	Orig.		+++
	Graft I	+++	+++
F10	Orig.		+++
	Graft I	+++	+++
	" II		+++
W1	Orig.	+++	
	Graft I	++	++
	" II		+
W4	Orig.		+++
	Graft I	+++	+++
R.W1	Orig.	++	
	Graft I	+++	+++

* + = retardation of growth, ++ = arrest of growth, +++ = regression (tumor not palpable).

TABLE VIII. Comparison of Degree of Responsiveness of Transplanted Mammary Tumors to Various Hormonal Influences.

Treatment	No. of strains tested	Degree of response* 0	+	++	+++	% responsive	Kind of response
Hypex	25	2	8	5	10	92	Inhibit.
Ovex	22	5	7	4	6	78	"
Androg.*	27	8	3	10	6	70	"
Adrex	13	7	5	0	1	46	"
MtT (MtH)	10	0	0	1	8	100	Stimul.

* Retardation of tumor growth in males.

a remarkable "practical" homozygocity of the $W/_{Fu}$ strain. Transplantation data clearly indicate the autonomy of the $W/_{Fu}$ tumor strains.

Malignancy and latency periods are independent features. Fibroadenomas are benign tumors, carcinomas are malignant, yet both can grow fast or slow, both can be hormone dependent or independent, both progress in the course of passages with retention of basic features.

Experiments of Clifton *et al.* (to be published) indicate that growth of mammary glands is maintained by MtH (given in form of MtT grafts) in absence of gonads and adrenals and that cortisol markedly enhances secretion but not growth of the mammary gland. The present experiments point to a similar role of MtH with respect to growth of MT.

Summary. 1. Hormone dependence of original (spontaneous, radiation- and 3-methylcholanthrene induced) mammary tumors were compared with first and second generation grafts of the same tumor in fully histocompatible inbred strains of rats. 2. Almost all tumors, (carcinomas and fibroadenomas) were autonomous in that they grew in normal hosts of both sexes, but most of them were hormone responsive. 3. A fair index of responsiveness is the degree of preferential growth of tumors in females. 4. Growth is retarded by ovariectomy and hypophysectomy and is resumed upon subsequent grafts of mammotropic tumors (administered to supply mammary gland stimulating hormones). 5. Progression appears invariable in the course of transplantations in the strains studied, but hormonal responsiveness in the first passages was almost identical with that of primary tumors. 6. Malignancy, hormone responsiveness, and growth rate vary independently. A slow growing fibroadenoma can be hormone independent and rapidly growing, while a carcinoma can be highly hormone responsive. These experiments support the hypothesis which considers the mammotrope of the pituitary and its hormones to be the main direct stimulant of the mammary gland and its tumors.

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2. ———, *ibid.*, 1960, v103, 643.

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