Relation of Mammotropes to Mammary Tumors. IV. Development of Highly Hormone Dependent Mammary Tumors.* (26152)

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There are two simple methods to produce mammary carcinomas in rats: administration of a chemical carcinogen (as 3-methylcholanthrene(1)) and ionizing radiation(2). The former, reported by Huggins(1), calls for repeated oral administration of the carcinogen. We found(3) that a subthreshold dose of this carcinogen produces mammary carcinomas in most rats whose mammary glands had been stimulated by mammotrope. Mammotropic hormone(s) alone did not produce mammary carcinomas. On the basis of this and other experiments (*cf.* 4) the theory was advanced that the hormone is a mere promoter or activator of neoplastic cells induced by a carcinogen, and that the altered cells can remain latent for long periods. Mammotropic hormone(s) not only revived the regressed or arrested mammary carcinomas following hypophysectomy but also brought about many new mammary carcinomas in the same hypophysectomized host(5).

In earlier studies nearly 80% of the mammary carcinomas induced by repeated administrations of 3-methylcholanthrene were found to be hormone dependent in the original passage(5) but upon successive transplantations rapidly gave rise to less and less hormone responsive autonomous variants. It is probable that when a tissue or organ is subjected to an intensive carcinogenic treatment numerous cells undergo malignant transformation, and, upon transplantations, the host selects the more malignant (autonomous) variants. We postulated that by reducing the amount of carcinogen and increasing that of the stimulating hormones, it may be possible to develop a much needed highly hormone dependent and relatively stable tumor strain.

Materials and methods. The 6 mammary carcinomas studied originated in the mammary tumor induction experiment by a combined treatment of a subcarcinogenic dose of 3-methylcholanthrene (10 mg) and a grafted functional mammotropic tumor (3, *cf.* Table III). Tumor transplantations were made by injecting 0.1 ml of tumor mince subcutaneously into highly inbred Wistar/Furth (W/Fu) rats, in which the tumors were induced. Mammary carcinoma mince was injected into the right inguinal mammary gland region and mammotropic tumor (Strain W2,(6)) in the back of the neck. The recipients were approximately 2 to 3 months of age. For estrogen treatment a pellet containing 0.5 mg diethylstilbestrol (DES) fused with 9.5 mg cholesterol was implanted subcutaneously into the back.

Results and comments. Five induced mammary carcinomas (W-5, 7, 9, 12, 16) were transplanted into W/Fu rats of both sexes. When 4 to 9 months after transplantation it was found that these tumors would not grow in either normal female or male rats, the rats were divided into 3 to 5 groups and were treated with DES or mammotropic tumor (to administer mammotropic hormone(s)), one group remaining untreated (control) as indicated in Table I. Treatment was initiated from 0 to 262 days after the mammary tumor transplantation.

With mammary carcinoma strain W-9, 3 successive sub-passages were made. All showed high mammotropic hormone dependency. None of the 5 strains could be transplanted to normal male rats and only 2 of 50 grafts (4%) were successful in untreated females. Transplantation was successful in 3 of 31 females (9.6%) treated with a DES pellet, while 50 of 56 females (89%) and all of 7 males that received mammotropic tumor developed a tumor.

In one of 6 carcinoma-bearing rats in which mammotropic tumor was completely resected, the mammary carcinoma promptly regressed

* This investigation was supported by contract with C.C.N.S.C. and grant from N.C.I.

TABLE I. Mammotropic Hormone Dependency of Mammary Carcinoma Induced by a Subthreshold Dose of 3-Methylcholanthrene and Mammotropic Hormone(s).

Mammary carcinoma strain	Passage	Days, graft to treatment	♀ Normal	♀ + DES†	♀ + Mammotropic tumor	♂ Normal	♂ + Mammotropic tumor
W-5	I	262	1/6*(321)	1/6 (321)	5/6 (322)	0/4	4/4 (330)
W-7	I	220	0/5	0/6	4/5 (278)		
W-9	I	143	0/7	1/6 (235)	5/6 (199)	0/5	
	IIa	17	0/10		14/14 (61)	0/4	
	IIIa	0	0/6		6/6 (36)	0/6	
	IIb	0	0/3		6/6 (120)		3/3 (122)
W-12	I	113	1/6 (197)	1/6 (197)	5/6 (181)		
W-16	I	0	0/7	0/7	5/7 (71)	0/2	
Total			2/50 = (4.0%)	3/31 = (9.6%)	50/56 = (89%)	0/21	7/7 = (100%)

* No. of takes over No. of rats inj. Numbers in parentheses indicate mean latent periods, in days.

† DES, 0.5 mg, in pellet subcut.

and never recurred, thus manifesting complete mammotropic hormone dependency.

Earlier experiments indicated that estrogen facilitates mammary carcinoma growth by way of stimulating mammotrope(6). The difference between DES and mammotropic tumor treatment is best explained by differences in mammotropic hormone level of the treated animals.

The latency of mammary carcinomas varied with time of mammotropic tumor graft. A comparison of the passages of the mammary carcinoma strains indicates a direct relation between time of mammotropic tumor graft and mammary carcinoma latency. Delay in mammotropic tumor graft prolonged appearance of grafted mammary carcinoma. When mammary carcinoma strain W-9 graft was followed immediately by mammotropic tumor graft, mammary carcinoma appeared in 36 days. But when mammotropic tumor graft was delayed by 17 and 143 days, mammary carcinoma did not appear until after 61 and 199 days, respectively. Tumor cells can remain latent for as long as 262 days and be brought to rapid proliferation by mammotropic hormone(s) (Table I).

Pituitary tropic hormones do not destroy cells but merely regulate their numbers. Correspondingly, hormone dependent tumor cells are not destroyed in histocompatible hosts. They can remain dormant in glands made hypoplastic by inhibitory hormones or by deprivation of stimulating hormones.

Dependent tumors are usually slow and autonomous tumors fast growing. Both latency and rate of growth depend on degree of hormonal stimulation.

The microscopic appearance of a highly hormone dependent tumor of this series showed slight morphologic "dedifferentiation." The tumors were well differentiated adenocarcinomas. The tumor cells and their nuclei were fairly uniform in size and shape, and there was but slight hyperchromatophilia and loss of polarity.

Summary. 1. Highly hormone dependent mammary tumors were induced in rats by a combined treatment of a subthreshold dose of 3-methylcholanthrene and mammotropic hormone(s) (administered by grafts of a functional mammotropic tumor). 2. Growth of these mammary tumors was dependent upon continuation of stimulation by mammotropic hormone(s). 3. Grafts of the mammary tumors remained latent up to 262 days, growing only after mammotropic hormone(s) was administered. 4. Upon resection of mammotropic tumor, a primary mammary tumor promptly and completely regressed; 5 other primary tumors in rats carrying functional mammotropic tumors grew progressively.

The authors acknowledge the technical assistance of Mary Campbell.

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Received August 30, 1960. P.S.E.B.M., 1960, v105.

Starch Gel Electrophoresis of Lactic Dehydrogenase from Rat Kidney.* (26153)

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Markert and Moller(1) and Tsao(2) have reported methods for localization of dehydrogenase activity after starch gel electrophoresis. Markert and Moller employed neotetrazolium as the terminal electron acceptor in a medium containing substrate, cofactor, methylene blue, diaphorase and hydrazine while Tsao employed the same tetrazolium, omitted diaphorase and replaced hydrazine with cyanide. Use of neotetrazolium necessitated the application of the incubating medium in the form of an agar(1) or starch gel(2) overlay to obtain anaerobic conditions and also required long periods of incubation.

This report describes an improved method for demonstration of lactic dehydrogenase (LDH) after starch gel electrophoresis based on the use of the more sensitive tetrazolium, Nitro BT, and substitution of phenazine methosulfate (PMS) for exogenous diaphorase. In addition, the standard incubating medium was varied in an attempt to characterize more closely the LDH fractions obtained from homogenates of kidneys of adult rats. Also, a technic is described for demonstration of diphosphopyridine nucleotide (DPN) diaphorase after starch gel electrophoresis.

Methods. Whole rat kidneys, homogenized in an equal volume of water, were frozen and thawed several times and centrifuged to remove tissue debris. A portion of the supernatant from each kidney was placed on a filter paper strip, inserted into a starch column, and subjected to electrophoresis for 6 hours(3,4).

Following electrophoresis, each column was sliced into 5 strips. One strip was incubated in the standard medium containing sodium lactate, 0.1M; DPN, 0.3 mg/ml; PMS, 20 μg/ml; Nitro Bt, 0.5 mg/ml; potassium cyanide, 0.1M; and phosphate buffer, 0.25M (pH 7.4) at 37° for one hour. In initial determinations, exogenous diaphorase was employed in the standard medium but this was subsequently replaced by PMS. Of the remaining strips, one each was incubated in a modification of the standard medium in which either DPN, PMS, or both DPN and PMS were omitted. DPN diaphorase activity was revealed by incubating a strip in a medium containing reduced DPN, 1.0 mg/ml; Nitro BT, 0.5 mg/ml; and phosphate buffer, 0.5M (pH 7.4). Malic dehydrogenase activity was demonstrated by substituting sodium malate for sodium lactate in the standard medium.

Results. Five distinct bands exhibited LDH activity when the column was incubated in the standard substrate (Fig. 1). Four of the bands migrated toward the anode while one band was on the cathodal side of the origin. When the column was incubated in the medium from which DPN had been omitted, bands 1, 4, and 5 could not be demonstrated, while either band 2 or band 3 was enzymatically active. In the absence of

* This work was supported in part by Univ. of Michigan Cancer Research Inst.

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