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Relation of Mammary Tumors to Mammotropes.* I. Induction of Mammary Tumors in Rats. (25621)

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It has been well established that estrogens and the pituitary gland play a major role in development of mammary gland(1) and in maintenance of growth of mammary tumors (2,3). Our studies point to a cell in the pituitary gland, named mammotrope, which produces a hormone (mammotropin) which is the direct stimulant of the mammary gland(4). Estrogens act indirectly by stimulating mammotropes(4,5). This concept is strengthened by observations described in this and the following papers(6,7), which point to the role of mammotropin in initiating as well as maintaining mammary tumors.†

Methods. Tumor induction. Mammary tumors were induced by 3-MCA in 2 highly inbred strains following Huggins' scheme(3). The rat strains used will be designated F_{Fu} and W_{Fu} following accepted terminology by mouse geneticists. The Fisher strain (F_{Fu}) was obtained from Dr. Wilhelmina Dunning in 1955; the Wistar strain (W_{Fu}) was a partially inbred strain purchased prior to 1945. Both were rigidly inbred and are homozygous from standpoint of transplantation. Breeding and care of rats and features of the W_{Fu} strain have been described(8). *Exp. I.* Four groups of F_{Fu} females (F-I, III, IV and V), one group of castrated F_{Fu} males (F-II), and one group of W_{Fu} females were treated with 3-MCA as indicated in Table I. Two weeks after the last treatment (112 days after start of 3-MCA feeding), a 1 mg pellet of DES was implanted s.c. in 10 rats of the F-II group. *Exp. II.* The influence of MtH on induction of mammary tumors was studied, using functional MtT as a source of this hormone(5). MtT (Strain W6) was grafted s.c. into rats of Groups W-I and W-III, 2 weeks prior to administration of 3-MCA. When a mammary tumor was detected in these rats, a biopsy was made and the tumor resected; parts of it were sectioned and parts transplanted in normal and conditioned rats, as described in the following report(6).

Results. Results of Exp. I are summarized in Tables I and II. Mortality due to toxicity of the carcinogen, indicated by severe fatty degeneration of the liver, was heavy only in Group F-V to which 3-MCA was given 6 times weekly. MT incidence was higher in 50 day old F_{Fu} rats (88 to 100%) than in those 83 days old (50%). All tumors were malignant with one exception, a fibroadenoma in a castrated male that was also given DES. One sarcoma was found in an F_{Fu} female that had multiple carcinomas at death 235 days after treatment. Most animals usually bore more than one tumor; the largest number in one rat was 13. Tumors arose in all mammary glands, perhaps most frequently in those of the thoracic region.

All W_{Fu} 3-MCA treated females developed MT within 5 months, although they received less carcinogen than F_{Fu} rats. Correspondingly, latency periods were shorter in W_{Fu} rats (Table II). All tumors in W_{Fu} rats were carcinomas.

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† *Abbreviations*: MT = mammary tumor; Mt = mammotrope; MtH = mammotropic hormone = mammotropin; MtT = mammotropic tumor; 3-MCA = 3-methylcholanthrene; DES = diethylstilbestrol.

TABLE I. Tumor Induction by Intragastric Instillation of 3-Methylcholanthrene in 10 mg doses.

Group No., sex	F-I ♀	F-III ♀	F-IV ♀	F-V ♀	F-II ♂	W-IV ♀
No. of rats:						
At start	17	22	27	23	20	18
Loss*	3	1	1	20	1	0
Rat						
Age (days)	83	51	50	70	83	56
Mean wt (g)	138	103	100	134	193	110
Treatment:						
Frequency, wkly	2×	2×	2×	6×	2×	2×
Duration, wk	14½	14½	13	6	14½	9
Total dose, mg	290	290	260	350	290	180
Tumor incidence:						
Mammary tumors:						
" adenocarcinoma	7 (50%)‡	21 (100 %)	22 (88 %)	2 (66.6%)	0	18 (100%)
" fibroadenoma	0	0	0	0	1 (20 %)	0
Squamous carcinoma:						
Periauricular	4 (50%)	14 (66.7%)	15 (62.5%)	1 (33 %)	10 (52.6%)	0
Skin	0	1 (11 %)	4 (25 %)	0	1 (33 %)	0
Mouth	1 (20%)	0	2 (10.5%)	0	1 (7.7%)	0
Leukemia	0	3 (20 %)	3 (11.5%)	0	1 (20 %)	0
Rats with tumor	9	21	25	3	10	18
Rats without tumor†	5	1	1	0	9	0

* No. dead before appearance of first tumor.
† Surviving latency period of first tumor.
‡ % calculated on basis of number of rats surviving first appearance of a particular tumor.

Incidence of squamous carcinomas of the periauricular sebaceous gland was 33 to 66% in $F/_{Fu}$ rats of both sexes. Other squamous carcinomas occurred in different parts of the body; 4 of these were in the mouth. None were found in $W/_{Fu}$ rats (Table I). Six leukemias developed in $F/_{Fu}$ female rats at 120 to 194 days of age, and 2 of these preceded all other tumors. No untreated controls were set up, but similar tumors were not seen at comparable ages in our breeding and ageing colony and stock of $F/_{Fu}$ and $W/_{Fu}$ rats.

Results of Exp. II are summarized in Table III. A single dose of 3-MCA produced mammary carcinomas only in animals whose mammary glands had been stimulated by MtT. Neither MtT nor 3-MCA alone was carcinogenic as here applied.

Comments. Induction of MT by intragastric instillation of 3-MCA, originated by Shay *et al.*(9) and perfected by Huggins *et al.*(3), is hereby confirmed in 2 highly inbred strains of rats. Similar tumors were not found in these strains at comparable ages, but MT and leukemia are known to occur in older animals of these strains at a much lower frequency (8). The spontaneous mammary tumors in

TABLE II. Latency Periods* of 3-MCA Induced Tumors.

Group	F-I	F-III	F-IV	F-V	F-II	W-IV
Mammary tumors	113-169 (131)	96-165 (149)	90-160 (116)	92	248†	72-154 (101)
Squamous Ca:						
Periauricular	175-200 (187)	147-255 (185)	167-263 (211)	123	140-392 (227)	
Skin		216	220-256 (235)		314	
Mouth	261		199-205		249	
Leukemia		194 (194)	120-168 (148)		249	

* Extremes with mean in parenthesis.
† Fibroadenoma.

TABLE III. Cocarcinogenesis with Mammotropes and 3-Methylcholanthrene.

Group	W-II	W-III	W-I
Treatment	3-MCA	MtT	3-MCA + MtT
No. of rats	8	8	7
MT, No. and type	1 fibroad.	1 fibroad.	6 ca., 1 fibroad.
Latency MT (days)	213	158	159-227 (170)

these and other strains of rats are usually benign. No mammary carcinomas were produced in male rats, pointing to a strong hormonal factor (*cf.* Huggins, 3) which as described in the next paper, is related to mammotropes.

Numerous squamous carcinomas and leukemias occurred in both sexes of the $F/_{Fu}$ strain, but not in the $W/_{Fu}$ strain. This may be a strain difference, possibly of genetic nature, but a confirmatory experiment is needed because total dose of carcinogen given to $W/_{Fu}$ rats was smaller and duration of treatment shorter.

An orientation experiment was performed to test our hypothesis that stimulation of the mammary gland by MtH promotes carcinogenesis. The findings strongly support this postulate. Observations of others (*cf.* Dao *et al.* 10) indicating that this hydrocarbon is concentrated in the mammary gland (as well as in fat) are in line with our hypothesis. We suppose that stimulation of the mammary gland by MtH enhances ability of the gland to trap the hydrocarbon. Male breasts also concentrate the carcinogen(10). However, they contain relatively few glandular elements but abundant fat where the carcinogen is located almost exclusively. This explains why they do not develop MT. In pregnant and lactating rats in which tumor induction rates are much higher than in virgin females(10), mammary glands are under high physiologic MtH stimulation, with intracellular as well as intraacinar concentration of lipid (milk). According to our hypothesis, male breasts stimulated by MtH and treated with 3-MCA should also develop MT (Exp. in progress).

Induction of leukemia in non-inbred W rats has already been described by Shay *et al.*(11), using 20-MCA in smaller doses over very long periods.

Summary. 1. Mammary carcinomas occurred in 50% to 100% of 64 $F/_{Fu}$ female rats surviving 90 to 96 days after initiation of intragastric instillation of 3-MCA twice weekly in 10 mg doses, and all of 18 $W/_{Fu}$ rats similarly treated, after 72 days of treatment. In castrated $F/_{Fu}$ male rats similarly treated, no mammary carcinomas developed. 2. Squamous carcinomas arising in periauricular sebaceous glands developed in about one half of both male and female $F/_{Fu}$ rats surviving 123 days, and in none of the $W/_{Fu}$ rats. Similarly, squamous carcinomas of skin and oral mucosa occurred in approximately 12% of $F/_{Fu}$ rats of both sexes and none in $W/_{Fu}$ rats. 3. Leukemias, including lymphoma (about 9%) developed in $F/_{Fu}$ rats and none in $W/_{Fu}$ rats. 4. A single dose of 3-MCA or MtH alone failed to produce mammary carcinomas in 8 $W/_{Fu}$ rats, while 6 of 7 rats receiving both, developed mammary carcinomas.

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