Relation of Mammotropes to Mammary Tumors. V. Role of Mammotropes in Radiation Carcinogenesis.* (26796)

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It has been well established that estrogenic hormones and the pituitary gland play a major role in development of mammary gland, in initiation and maintenance of growth of mammary tumors, and that in adults these actions of estrogens upon the mammary gland are mediated by a pituitary cell called mammotrope (Mt), which secretes mammary gland stimulating and growth promoting hormones(1). Enhancement of induction of mammary tumors (MT) by these hormones (MtH) in rats x-rayed with 200r or 400r over entire body, from 9% to 76%, within 7 months has been reported(2). It seemed desirable to confirm and extend this work using smaller doses of x-rays which alone will produce no MT within 7 months.

Materials and methods. The strain of rats (W/Fu) used and general procedures have been described(2,3). The rats were 2 to 3 months old when irradiated. The factors of x-irradiation were: 250 Kvp; 30 ma; filtration 1/4 Cu. 1.0 Al; distance 50 cm; air dose 150r/min. The rats were irradiated under light Nembutal anesthesia. As a source of steady supply of a natural MtH, transplantable mammotropic pituitary tumors (MtT) (Strains W-5 and W-6) were used. Strain W-5 was induced by radiation(2). Strain W-6 occurred in an old W/Fu female. MtT was grafted subcutaneously on back; when it reached about 3 cm in greatest diameter, most of it was resected to prevent death from a large tumor. The rats were sacrificed when a MT was palpable, and the MT and several mammary glands were examined microscopically. The ovaries of a small number of rats were removed 80 days after irradiation when the grafted MtT was not yet palpable. The experiments were terminated 7 months after irradiation when the rats were about 9 to 10 months old.

* Supported by Atomic Energy Commission and Nat. Cancer Inst.

Results. No MT occurred in 20 normal controls, in 23 rats treated with MtH alone and in 17 rats given 50r alone. Only 1 of 16 rats given 150r alone developed a MT (adenocarcinoma) at 202 days after irradiation. In contrast, 22 of 32 rats receiving both MtH and x-rays developed MT: 8 (53%) received 50r and 14 (82%) received 150r (Table I).

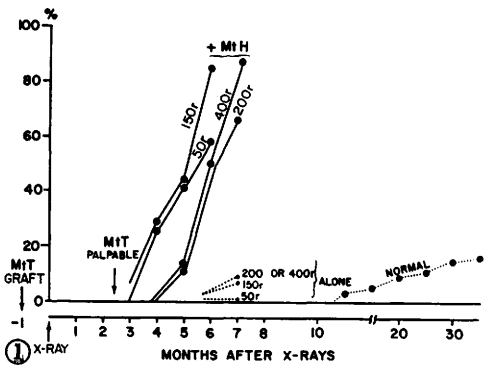
Fig. 1 shows the cumulative incidence of MT, including previous findings with 200r and 400r. The figure shows that onset of MT was slightly delayed when larger radiation doses were given. This apparent paradox can be readily explained. As the figure shows, the grafted MtT was also irradiated and it suffered from the large doses, its latency was prolonged and, consequently, these rats received less hormone than those given smaller doses of radiations. In general, MT became palpable earlier in those rats in which the MtT, that supplied the hormone, appeared sooner. Fig. 1 also shows that the spontaneous incidence of MT in females of this strain was 16% in 196 females observed up to 32 months of age and that all spontaneous tumors were fibroadenomas(3).

The types of MT and their latency are shown in Table II. Most MT induced by radiation alone were fibroadenomas; most induced by MtH plus radiation were adenocarcinomas. In general, induced carcinomas appeared earlier than fibroadenomas, as was already noted by Bond *et al.*(4).

Transplantation assay for autonomy. Most MT induced with 3-methylcholanthrene were

TABLE I. Mammary Tumor Induction by X-rays and MtH in Female W/Fu Rats.

Treatment	No. in group	MT	
		No.	%
None	20	0	
MtH	23	0	
50r	17	0	
150r	16	1	6.2
50r + MtH	15	8	53
150r + MtH	17	14	82



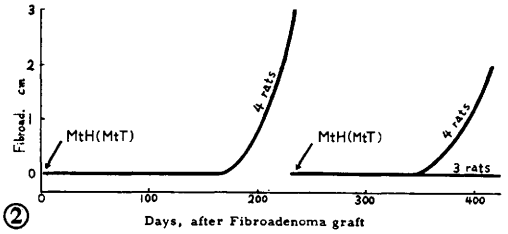


FIG. 1. Cumulative incidence of MT induced by X-rays and MtH in female W/Fu rats.

FIG. 2. Dependence of growth of grafted fibroadenoma on MtH.

autonomous but responsive to hormones(5). Tumors induced with large doses of this carcinogen, transplanted in successive passages, soon gave rise to fully autonomous variants; whereas, those induced with a single small dose of the carcinogen plus MtH remained fully MtH dependent(6). In light of this information, 3 radiation- plus MtH-induced tumors were tested for hormone responsiveness. All 3, a fibroadenoma and 2 adenocarcinomas, were found to be highly hormone responsive (Table III). They grew in all MtH-treated female rats, in few normal females, and in no normal males. The latent periods of grafted fibroadenomas were about 6 months and of the adenocarcinomas 2 months.

TABLE III. Transplantation of MT Induced by X-rays and MtH in W/Fu Rats.*

Tumor grafted	Recipient ♂	♀	♀ + MtH
Fibroadenoma W-1	0/4	1/8	4/4
Carcinoma W-19	0/5	1/8	4/4
" W-20	0/5	2/11	6/6

* Grafts from original tumors; sub-passages are in progress.

The dependence of growth of the fibroadenoma on MtH is shown in Fig. 2. This tumor was grafted in 11 female rats, 4 of which received MtH (by MtT graft) immediately after the graft, and tumors became palpable in the latter after about 180 days. About 230 days after the fibroadenoma graft, MtH was administered to 4 of the 7 remaining rats; all of these developed palpable tumors 140 days later. The 3 rats not receiving MtH were sacrificed 405 days after graft and carefully autopsied. No tumors were found. This finding is similar to that with 3-methylcholanthrene-induced hormone-dependent carcinomas which could be fully controlled by ovariectomy or hypophysectomy and resumed vigorous growth after administration of MtH(5). The present work indicates that MT induced by a combination of MtH and radiation can also be highly hormone (MtH) responsive.

TABLE II. Type and Latency of MT Induced by X-rays and MtH in Female W/Fu Rats.

Treatment	No. with MT/No. in group	Type of tumor Carcinoma	Fibroad.	MT latency after x-ray (month) 3	4	5	6	7
150r	1/16	1						1
200r*	1/11		1					(1)‡
400r*	1/11		1					(1)
MtH + 50r	8/15	6	2		3	2 (1)	1 (1)	
MtH + 50r + Ovex§	1/4	1				1		
MtH + 150r	14/17	11	3	2	3	1 (2)	5 (1)	
MtH + 150r + Ovex§	2/4	1	1					1 (1)
MtH + 200r*	6/9	4	2			(1)	3	1 (1)
MtH + 400r*	7/8†	2	6			1 (1)	(3)	1 (2)

* Reported earlier(2) without specification as to latency and type.
† One of these rats had 2 tumors, a fibroadenoma and an adenocarcinoma, listed separately.
‡ Figures in parentheses are fibroadenomas.
§ Ovex = bilateral ovariectomy, performed 80 days after x-irradiation.

TABLE IV. Effect of Ovariectomy on MT Induction by X-rays and MtH.

Treatment	No. of rats	No. with MT	Tumor latency (days)
X-ray*	6	0	
X-ray + MtH	6	3	90, 125, 145 (120)‡
X-ray + Ovex†	8	0	
X-ray + MtH + Ovex	8	3	145, 210, 210 (188)

* 50r or 150r, over entire body.
† Bilateral ovariectomy, performed 80 days after x-irradiation.
‡ Figures in parentheses indicate mean latency.

In a small experiment, 8 irradiated plus MtT grafted rats were ovariectomized 80 days after irradiation when MtT was not yet palpable. Ovariectomy seems to slow down MT development and may also prevent MT formation in some rats (Table IV).

Discussion. Two strong factors have long been recognized in mammary tumorigenesis: estrogenic hormones which exert their influence by way of the pituitary Mt(1) and carcinogens which cause a permanent modification of the mammary epithelium. Earlier studies have shown that ionizing radiations stimulate Mt(2), the supplier of MtH that promotes MT development. Thus, radiations have a double action: they "cancerize" mammary epithelium and stimulate its promoter, Mt.

The relative role of hormones and carcinogens in induction of mammary tumors has been fully discussed elsewhere(7). The observations here presented suggest that small doses of radiations can create latent neoplastic cells and that these cells form progressively growing tumors only when the hormonal stimulus (MtH level) is elevated. Thus, it seems that the dependent tumorous mammary epithelium has a greater sensitivity to MtH than the normal epithelium. A similar heightened sensitivity to estrogens of dependent tumorous pituitary Mt, in comparison to normal Mt, is indicated by studies of Clifton(8) by means of grafting these cells beneath the kidney capsule. Formation of such dependent neoplasms is a unique type of carcinogenesis in which tumor formation is brought about by a mild permanent modification of the cell in presence of elevated levels of its stimulant.

Experiments on the degree of mammary gland stimulation by MtH, as indicated by incorporation of tritiated thymidine, are in progress in our laboratory. B. H. Sells and B. Messier found that under stimulation of MtH (by a small graft of MtT), the mammary tissue incorporates twice as much tritiated thymidine per gram as unstimulated mammary tissue and total uptake of tritiated thymidine in stimulated glands is nearly 10-fold normal (unpublished data).

These and other studies(7) led us to the generalizations that physiologic doses of hormones are not carcinogens and that small, so-called subcarcinogenic doses of x-rays as well as chemical carcinogens and virus can produce many latent neoplastic cells and that the latter can be activated by proliferative stimuli of which hormones are outstanding.

How long latent tumor cells can survive and what the relation is between their numbers and the dose of radiation remains to be studied. Conceivably, there is no threshold for the "carcinogenic" alteration which may be "linear" but it is unlikely that tumor formation follows the same "linearity." The work discussed indicates that to bring about a tumor formation, promoting factors, such as MtH, are needed. The availability of the latter and the magnitude of the radiation damage determine the formation of tumors by altered cells.

Summary. 1. X-rays (50r) and mammotropic hormones (administered in form of isologous functional mammotropic pituitary tumor grafts) alone produced no mammary tumors in female W/Fu rats within 7 months of treatment. In contrast, 53% rats treated with both, developed mammray tumors. As with chemical carcinogen-induced tumors, administration of mammotropic hormones restored inhibition of mammary tumor development by ovariectomy. 2. Most mammary tumors induced by radiation plus mammotropic hormones were adenocarcinomas; a few were fibroadenomas. 3. Three induced tumors tested were found to be highly mammotropic hormone responsive: they did not grow in males and grew in females uniformly

only when stimulated with mammotropic hormones. 4. Grafted fibroadenoma cells remained latent in many rats until administered mammotropic hormones brought about their rapid growth. Administration of this hormone could be delayed as long as 7½ months. 5. It is concluded that radiations bring about an irreversible modification of some cells, depending on severity of the dose; mammotropic hormones are not carcinogens but, as promoters of mammary epithelium, promote carcinogenesis and growth of some tumors.

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Received June 19, 1961. P.S.E.B.M., 1961, v107.

Erythrocyte Survival Studies in Experimental Molybdenosis of Sheep.* (26797)

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The toxicity of a high molybdenum intake in the diet has been extensively studied. In cattle, the effects of toxicity include severe diarrhea, emaciation, loss of coat color and anemia. In sheep on similar intakes of molybdenum, the clinical picture appears to be principally associated with signs of a copper deficiency. The relationship between molybdenum and copper metabolism together with the influence of inorganic sulfate has been recently reported by Dick(1). It was shown that the symptoms of copper deficiency in sheep develop only in presence of increased molybdenum and sulfate in the diet. Similar molybdenum sulfate-copper relationships in cattle have been reported(2). Experimental molybdenosis has not been shown to influence hematology in sheep(3) or cattle(2) even though an anemia is associated with the natural condition in cattle.

This report presents data concerning the life span of erythrocytes in experimental molybdenosis of sheep using the direct glycine-2-C^{14} method. Erythrocyte life span for normal sheep using this method has been previously reported(4).

Materials and methods. Two female lambs (6161, 6170), one year of age and weighing 28 and 32 kg, respectively, were used. Each lamb was initially placed on a daily oral intake by capsule of 45.4 mg of molybdenum as sodium molybdate for 54 days. Since no clinical symptoms of toxicity appeared, daily intake was doubled for the next 35 days, during which time severe diarrhea developed. Daily intake was then returned to the original amount for 10 days. At this time, 100 μc and 150 μc of glycine-2-C^{14} were injected intravenously into lambs 6161 and 6170, respectively. Molybdenum administration was continued throughout the trial.

The procedures employed for determination of median survival time for erythrocytes in sheep have been reported(4,5). Median survival times ($t_{½}$), of the erythrocytes of the sheep in this study were estimated from their survival probability function, $p(t)$, the probability that an erythrocyte will have a survival time greater than t.

Plasma and liver copper levels were determined in the sheep at termination of the trial

* This work was supported in part by the Western Regional Project 35.