Influence of Repeated Doses of 3-Methylcholanthrene at Fixed Intervals on Breast Cancer in Sprague-Dawley Female Rats.* (27331)

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The breast tissue of virgin female rats of the Sprague-Dawley strain is exquisitely sensitive to the carcinogenic action of 3-methylcholanthrene (3-MC) when this hydrocarbon is administered by the oral route. Many of the factors which influence the carcinogenic response have been demonstrated by Huggins, *et al.*(1,2,3). Huggins, *et al.*(4) reported that a single feeding of 3-MC (66 mg/100 g rat) is optimal for induction of mammary cancer in female Sprague-Dawley rats, age 50 to 65 days. The observations to be reported demonstrate that multiple feedings of an *optimal* dose of 3-MC shorten tumor induction time and increase the number of tumors. The number of breast cancers so induced is an absolute function of the total dose of carcinogen.

Methods. 3-MC was dissolved in sesame oil, 20 mg/ml, and administered by gastric tube to female Sprague-Dawley rats according to the following regimen. The dose of hydrocarbon administered was 66 mg/100 g body weight.

Group 1. 3-MC, age 50 da: sesame oil, age 60, 70, 80 and 90 da.

Group 2. 3-MC, age 50 and 60 da; sesame oil, age 70, 80 and 90 da.

Group 3. 3-MC, age 50, 60 and 70 da; sesame oil, age 80 and 90 da.

Group 4. 3-MC, age 50, 60, 70 and 80 da: sesame oil, age 90 da.

Group 5. 3-MC, age 50, 60, 70, 80 and 90 da. All rats were housed in stainless steel cages in a lighted laboratory, T. 79 ± 2°F. Food consisted of commercial ration, lettuce twice weekly, and one orange per 12 rats daily. On the 25th day of the experiment daily palpation of each rat for the presence of mammary tumor was begun. All animals were killed after 90 days' observation. Tumors were excised and promptly weighed.

Results and discussion. The results of this experiment are presented in Table I. After a single feeding of the carcinogen 17 of 22 rats (77.3%) had mammary cancer in 90 days; mean tumor induction time was 67.2 days. Cancer was observed in all rats given 2 or more doses of 3-MC. Mean tumor induction time was 51.3 days after 2 doses and 42.8, 43.4 and 43.5 days after 3,4 and 5 doses, respectively. Maximal acceleration of mean tumor induction time was achieved with 3 doses.

A striking increase in number of breast cancers occurred as the total dose of 3-MC increased. This response (Fig. 1) was not a logarithmic function of total dose nor of the number of doses, but one of addition. No significant difference in mean tumor weights of the 5 treatment groups was observed.

Despite the high doses of hydrocarbon employed, no signs of toxicity were observed. Weight gains for all treatment groups were

TABLE I. Mammary Cancer Incidence and Number of Tumors in Female Sprague-Dawley Rats Given 3-Methylcholanthrene 66 mg/100 g by Gastric Tube at 10-Day Intervals.

No. rats	No. doses	Total dose (mg) avg	Mammary cancer (%)	Appearance of 1st palpable tumor (days)			Active centers,	△ B.W. (g) avg
				Mean ± S.E.	Range	Median	Mean ± S.E.	
22	1	106	77.3	67.2 ± 3.80	31-92	75	2.4 ± .41	+102
24	2	237	100.	51.3 ± 3.01	33-79	46	4.5 ± .47	+102
24	3	358	100.	42.8 ± 1.19	33-55	41	9.2 ± .76	+ 97
24	4	505	100.	43.4 ± 1.56	31-60	42	10.7 ± .9	+ 94
24	4	656	100.	43.5 ± 1.32	31-55	43	13.9 ± .96	+103

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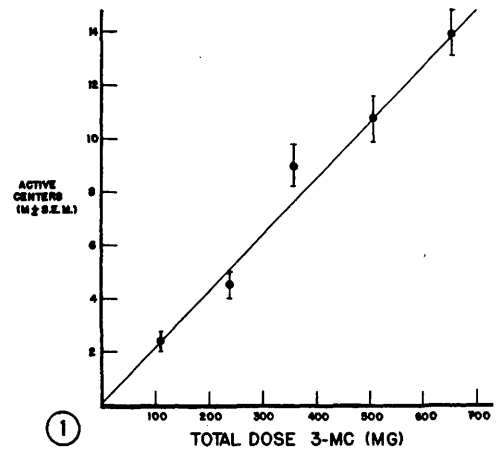

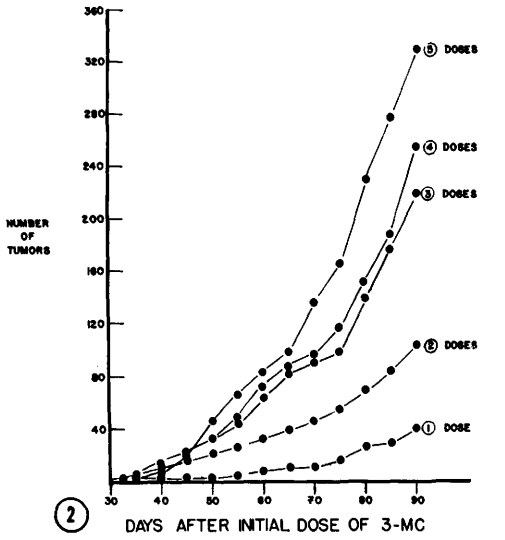

FIG. 1. No. of tumors (mean ± stand. error of mean)/rat, 92 days after initial feeding of 3-methylcholanthrene.

FIG. 2. Absolute No. of tumors palpated during observation period following initial feeding of 3-methylcholanthrene by gastric tube. No. of tumors at 90 days was determined at necropsy.

comparable and survival was 100%.

These observations provide no insight regarding the basic mechanism(s) of chemical carcinogenesis; they do, however, indicate that the number of tumors induced is a function of the number of doses of carcinogen, an effect which appears to be quantitatively additive. When given a single effective dose of 3-MC at 50 days of age, all female rats of this Sprague-Dawley strain will develop mammary cancer in time. What determines which cells will become neoplastic or why this carcinogen has a particular affinity for breast tissue is unknown. It is unlikely that any cell escapes damage to some degree. Increasing the number of doses of carcinogen apparently accelerates the neoplastic process by reenforcing the mutagenic effects of the initial dose causing more cells to become frankly neoplastic. The slope of the curves in Fig. 2 favors this interpretation, since the number of tumors observed is considerably higher than would be expected if each dose of carcinogen induced cancer *de novo*. Mean tumor induction time is a parameter which is measured by palpating an aggregate of neoplastic cells. A reduced tumor induction time may be an expression of the number of neoplastic cells rather than an actual decrease in time required for the neoplastic process.

The data presented here for 3-MC induced carcinogenesis appear to correspond in principle with those of Blum(5) for ultra-violet radiation-induced ear cancer and of Druckerey(6) for other chemical carcinogens.

Summary. 3-Methylcholanthrene, 0.66 mg/g rat, was administered by gastric tube to female Sprague-Dawley rats, age 50 days. This dose was repeated at 10-day intervals 1, 2, 3, or 4 times. Mammary cancer was observed in 77.3% of rats 90 days after a single dose and in 100% of the animals given 2 or more doses of carcinogen. Maximal acceleration of mean tumor induction time was achieved with 3 doses. The number of tumors observed correlated directly with the number of doses administered, a response which appears to be quantitatively additive.

1. Huggins, C., Briziarelli, G., Sutton, H., *J. Exp. Med.*, 1959, v109, 25.

2. Huggins, C., Grand, L. C., Brillantes, F. P., *Proc. Nat. Acad. Sci.*, 1959, v45, 1294.

3. Huggins, C., *Canad. Cancer Conf.*, 1961, v4, 81.

4. Huggins, C., Grand, L. C., Brillantes, F. P., *Nature*, 1961, v189, 204.

5. Blum, H. F., *J. Nat. Cancer Inst.*, 1959, v23, 319.

6. Druckerey, H., Ciba Found. Symp. on Carcinogenesis, London, 1958, 110.

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