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Lack of Correlation Between Dietary Level of Manganese and Growth Rate of Induced Fibrosarcomas.*† (19635)

DORSEY E. HOLTKAMP,† ROBERT M. HILL, AND ENID K. RUTLEDGE.

From the Departments of Biochemistry and Pathology, University of Colorado, Department of Medicine, Denver, Colo.

Recently we have demonstrated an effect of the level of dietary manganese on growth in young rats(1). Earlier Maxwell and Bischoff(2) reported that "no effect on tumor growth was noted" in 7 carcinoma-bearing rats that were given intraperitoneal doses of 8-10 mg/kg of Mn ($C_2H_3O_2$)₂ at 3- to 4-day intervals until 45-50 mg Mn/kg had been given. These authors observed, however, that there was less liquefaction of the tumors in the "dosed" animals in the later stages, and that "the occurrence of regressions was also somewhat higher in the dosed series. . . ." Ferguson, Templeton, and Patras(3) reported that "spindle cell sarcoma" transplants grew only about 75% as rapidly in rats on a diet from which the usual "salt-mixture" was omitted. This salt mixture contained small amounts of $MnSO_4$. Popoff (1941)(4) found that minute amounts of Mn, Li, and I_2 added together to the diets of tumor-bearing mice decreased the growth-rate of the

tumors to 1/3 or 1/4 of the usual rate. Thus we have one group of investigators(2) reporting "no effect" on tumor-growth from the intraperitoneal administration of extra manganese to tumor bearing rats; a second group (3) reporting a decrease in tumor-growth when the salt-mixture containing manganese is *omitted* from the diet; and a third group (4) reporting that minute amounts of Mn, Li, and I_2 *added* to the diet cause a decrease in the growth-rate of tumors in mice. In all except the first of these experiments, other factors in addition to Mn were varied.

In view of the inconclusive nature of these reports with respect to the growth of tumors, and in view of our demonstration(1) of the effect of the dietary level of Mn on the growth-rate of normal tissue, we have thought it worthwhile to investigate the growth-rate of methylcholanthrene induced-sarcomas of the thigh muscle of the rat with respect to the dietary Mn level.

Experimental. The rats used in these experiments were 30-60-day-old females from a strain that has been inbred at this institution for more than 20 years. In numerous earlier experiments we have shown that the injection of single doses of methylcholanthrene into the posterior muscles of the thigh by our technic produces fibrosarcomas in more than

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‡ Present address: Research Division, Smith, Kline, and French Laboratories, Philadelphia, Pa.

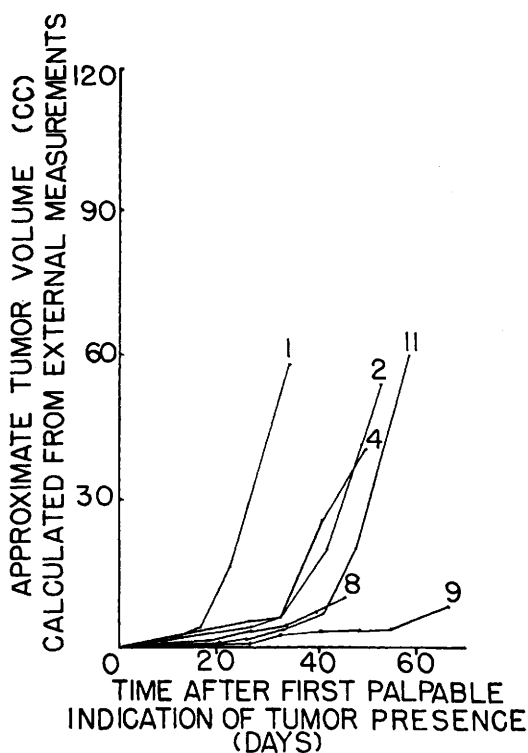


FIG. 1. Rate of growth of tumors in rats receiving no manganese supplement. Figures indicate individual rat numbers.

99% of the animals(5). Twenty rats were divided into groups of 6, 7, and 6. All the animals received a basic diet of Purina Fox Chow and distilled water *ad libitum*. This diet provides about 1 mg of manganese per rat per day.[§] Group I (6 rats) received this diet without supplement. Group II (7 rats) received, in addition, 10 mg Mn/rat/day; Group III (6 rats) received 40 mg Mn/rat/day. The Mn supplement, as $Mn SO_4$, was added to the drinking water. After the rats had been on the respective levels of manganese supplement for 30 days, the tumors were induced by one injection of 3 mg of methylcholanthrene (Eastman) in 0.25 ml of corn oil. The corn oil containing the methylcholanthrene was injected into the posterior muscles of the left mid thigh at an intramuscular depth of about 1 cm. Growth of the tumors was estimated by measuring the circumference of the thigh at the level of injection at approximately weekly intervals. From

[§] Purina Assay Charts.

this measurement, and from the assumption that the growing tumors were spheroids, the volume was calculated. For the animals used in these calculations the assumption of spheroid shape was borne out at autopsy.

Results. Results of these experiments are shown in Fig. 1, 2, and 3, which represent graphically the growth of methylcholanthrene-induced tumors in the thigh muscles of rats beginning with the first appearance of palpable growth. The diets contained 1, 11, and 41 mg of manganese per rat per day, respectively. The following comparisons were made between the results in the 3 groups: 1) The time between the injection of methylcholanthrene and appearance of the first palpable tumor, 2) the mean growth-rate from the time of the first appearance of palpable tumor growth to the end of the experiment, and 3) the growth-rate during the 2 weeks' period of maximum growth.

In the first comparison, mean values were so nearly the same that "no significance" was

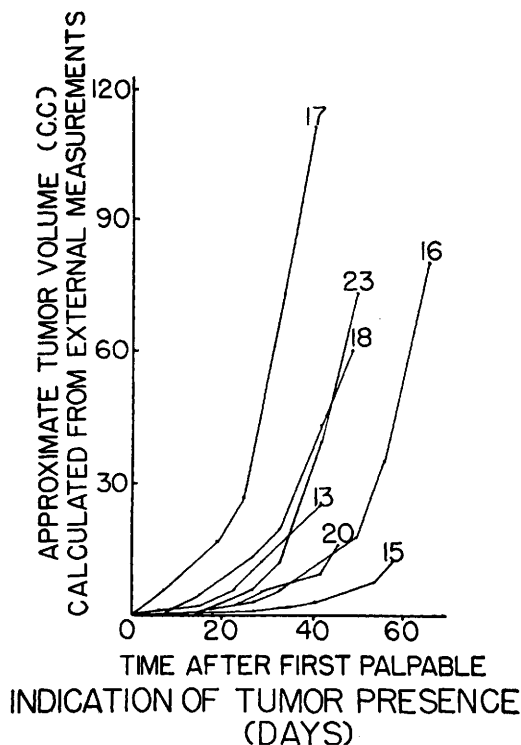


FIG. 2. Rate of growth of tumors in rats receiving 10 mg manganese supplement daily. Figures indicate individual rat numbers.

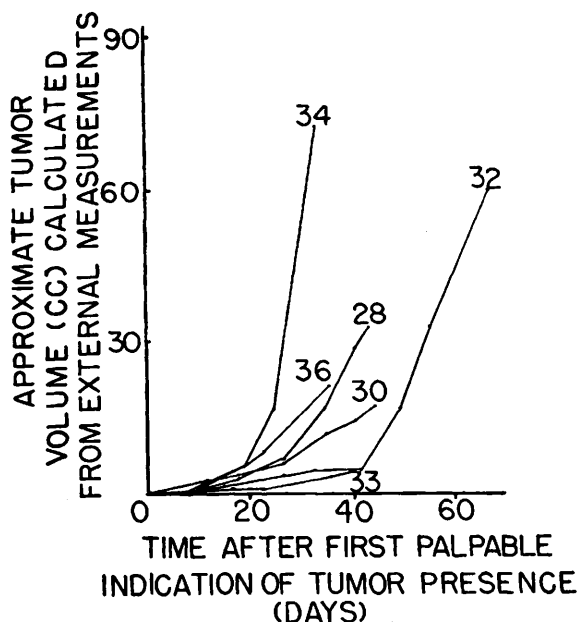


FIG. 3. Rate of growth of tumors in rats receiving 40 mg manganese supplement daily. Figures indicate individual rat numbers.

assumed without subjecting the data to statistical analysis. In the second and third, tests of significance of difference between means were applied, with correction for sample size. In every case the difference between means showed a value of "P" which ranged from 0.40 to 0.30 as a maximum and from 0.20 to 0.10 as a minimum.

Inasmuch as a tumor has been growing for an unknown time before it is first palpable, initial palpability as a measure of initial tumor-growth involves an unknown degree of error in the time factor. It is possible that if timing could be accomplished with greater precision, significant differences in the rate of tumor-growth in rats on different dietary levels of manganese might appear, both in the time between injection of methylcholanthrene and initial tumor-growth, and in the mean growth-rate of tumors. However, timing of initial growth does not affect the third comparison, the maximum growth-rate of the tumor.

From these experiments it must be concluded that, with the technics at our disposal, at dietary levels of manganese of 1, 11, and

41 mg per rat per day, the level of manganese intake has not been shown to influence the growth-rate of methylcholanthrene-induced sarcomas.

No difference in the degree of "liquefaction" of tumors was observed between the different groups, and no regressions occurred in any of the tumors.

Conclusions. At dietary levels of manganese of 1, 11, and 41 mg/rat/day, there is an apparent lack of correlation between level of dietary manganese intake and growth rate of methylcholanthrene-induced sarcomas of the thigh muscles of white rats.

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