

# The Effect of Diets Containing an Abundance of Milk, Liver, Riboflavin, and Xanthine on Methylcholanthrene Carcinogenesis\*

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(Received for publication April 25, 1946)

The numerous attempts to influence the development and growth of neoplasms by means of vitamins and other nutritional elements have been comprehensively summarized by other investigators (8, 9, 20). The experiment described here was stimulated by the observation that the dialyzed ground livers of cancer-resistant mice (JK), exhibit a higher level of xanthine dehydrogenase activity than do the dialyzed liver preparations of mice of a cancer-susceptible (C3H) strain (11). The mice of the JK strain were found also to be more tolerant to salicylaldehyde than were those of the C3H strain (21, 22). Xanthine dehydrogenase is a flavoprotein (2, 13), capable of oxidizing aldehydes as well as xanthines and found in high concentrations in milk and liver (2, 10, 12, 13, 19).

Axelrod and Elvehjem (1) showed that the xanthine dehydrogenase activity of rat liver is greatly reduced in riboflavin deficiency and that this decrease parallels the degree of the deficiency. The effect of feeding excessive amounts of the precursors of xanthine dehydrogenase on enzyme activity of the liver has not been determined.

This paper will be limited to a presentation and discussion of the results of feeding relatively enormous amounts of the dietary precursors of xanthine dehydrogenase to mice of the C3H strain. These mice have a relatively high susceptibility to spontaneous mammary carcinoma and methylcholanthrene tumors (7); and their livers, as compared with those of the resistant JK mice, exhibit a lower xanthine dehydrogenase activity (11). Xanthine, which is one of the normal substrates for xanthine dehydrogenase, was also included in this investigation because excessive amounts of either the substrate or uric acid, its oxidation product, inhibit the activity of xanthine dehydrogenase (10). It was thought that the inclusion of this substance in the diet might reduce the xanthine dehydro-

genase activity of the liver, thus creating a physiological state similar to that induced by a deficiency of either riboflavin or protein, precursors of xanthine dehydrogenase. The purpose of these experiments was to ascertain whether the incidence or latent period of methylcholanthrene tumors could be altered by such dietary procedures.

## METHODS AND MATERIALS

The basic experimental diet used contained the following ingredients: (a) 20 pounds of freshly ground pig liver; (b) 8 quarts of raw (unpasteurized) milk; (c) 50 pounds of Nurishmix;<sup>1</sup> and (d) 5 pounds Klim brand dried whole milk. These ingredients were thoroughly mixed and kneaded together, then dried and stored in an open container until fed to the mice. This diet will be referred to in this paper as the "Liver, Milk, Nurishmix," or L.M.N. diet.

Liver, in addition to other vitamins of the B complex, contains 10 to 30 mgm. per cent riboflavin (19), or about 10 to 30 times the amount found in muscle. The dried milk contains relatively high concentrations of casein and riboflavin. Raw milk contains free riboflavin and casein, and in addition an abundance of xanthine dehydrogenase (2, 13). Nurishmix contains dried buttermilk and meat scraps, and therefore appears to provide adequate protein and riboflavin even without the added liver and milk. Thus the L.M.N. diet was unusually high in protein (casein) and riboflavin. Some of the mice fed this mixture were given an additional quantity of riboflavin in milk substituted for drinking water. Xanthine was dissolved in the raw milk administered to one group of 11 animals.

Fifty-seven mice of the C3H strain were placed on the L.M.N. diet; 55 of the same strain and age (46 days) were placed on a diet of Nurishmix alone and used as controls. When both groups were 60 days of

\* This experiment has been made possible by grants from The Anna Fuller Fund and The Jane Coffin Childs Memorial Fund for Medical Research.

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<sup>1</sup> According to the manufacturers (Pratt Food Co.), Nurishmix contains dried buttermilk; beef scraps; wheat germ meal; rolled oats; molasses; iodized salt, 1%; and U.S.P. cod liver oil.

age they were injected subcutaneously on the right side with 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. Another 45 normal C3H mice were kept as a second group of controls in order to ascertain the normal growth rate of nontreated mice on the Nurishmix diet.

Three weeks after the injections of methylcholanthrene, the 57 experimental mice were divided into two main groups, as follows: I. Those continued throughout the experiment on L.M.N. diet—30 mice; and II. Those given fresh raw milk daily, *ad libitum*, in place of water—27 mice. This second group was further subdivided into 3 groups, as indicated in Table I. All

tively growing tumors up to that point. The data for the controls (on Nurishmix diet) are given on the solid line; the average data for all the experimental mice on L.M.N. diet (57 mice) are given on the short dash line; the data for those experimental mice not receiving a supplement of raw milk (L.M.N. diet alone) are given on the solid dot and long dash line; the data for those experimental mice (L.M.N. diet) receiving a supplement of fresh raw milk are given on the dotted line; and finally, the data for those mice receiving xanthine and riboflavin added to their supplement of fresh raw milk are given on the long dash line. It will be seen that all the curves for the ex-

TABLE I: SURVIVAL TIME WITH TUMORS

Data on (a) group of mice used; (b) diet for several groups; (c) No. of mice in each group and average survival time for various groups following appearance of induced fibrosarcomas.

Group	Diet	No. of mice	Survival time, days
A. Control (injected MCA).....	Nurishmix + water	55	36.0
A. Normal (non injected).....	Nurishmix + water	45	
B. Experimental (injected MCA).....	L. M. N. diet	57	27.5
I. L. M. N. diet alone and water.....		30	27.0
II. L. M. N. diet + raw milk.....		27	28.0
(a) Raw milk alone.....		8	29.7
(b) Raw milk + riboflavin*.....		8	27.7
(c) Raw milk, plus xanthine†, plus riboflavin.....		11	27.0

\*10 mgm. per 100 cc.  
†3.3 mgm. per 100 cc.

the mice that were given raw milk daily drank more liquid than those given water. The average milk intake per mouse per day was 3.5 cc., representing an intake of 0.35 mgm. of riboflavin per mouse in the cases where this was added; this is a huge dose for a mouse, and is approximately equivalent to 15 gm. of riboflavin per day for a 75 kgm. man. When mice on such a regime were examined at autopsy under near-ultraviolet light, the entire intestinal tract exhibited a brilliant yellow fluorescence from the high concentration of riboflavin.

Biweekly determinations of body weight and examinations for nodular masses at the site of injection were made. When a growing tumor became obvious, the body weights of these mice were compared with the body weights of those in the same groups still free of induced tumors. Growth rate of the tumors and survival time of the mice were determined and compared to similar data for the normal controls.

RESULTS

The mice in the various groups exhibited uniform growth rates. The average latent periods for the various groups are given in Fig. 1. In this graph the data are given cumulatively; that is, the data at any given age period included all mice that had developed ac-

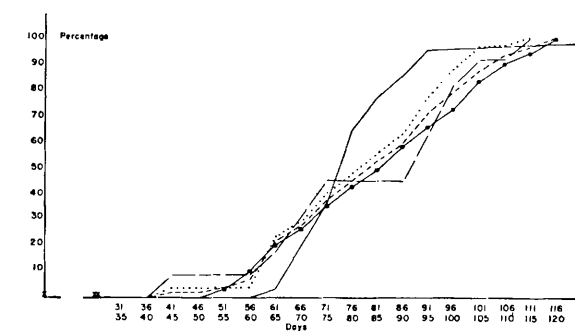


FIG. 1.—Average latent periods. The data are given cumulatively. Percentage of tumors on vertical line; average latent period on base line; data for controls on solid line; for all experimental mice on L.M.N. diet on short dash line; for those experimental mice not receiving a supplement of raw milk (L.M.N. diet alone) on solid dot and long dash line; for experimental mice receiving a supplement of fresh raw milk on dotted line; and for those receiving xanthine and riboflavin added to supplement of fresh raw milk on long dash line. x indicates injection of methylcholanthrene; xx, raw milk riboflavin supplement started.

perimental mice tend to group together and all deviate but slightly from the curve obtained for the control mice kept on Nurishmix diet alone.

Seven mice out of a total of 57 on the L.M.N. diet developed progressively growing tumors before any of

the 55 control mice did. That is, the L.M.N. diet appeared at first to cause an earlier incidence of induced tumors. At 86 to 90 days following the injection of methylcholanthrene, 35 of the 57 mice on L.M.N. diet had developed tumors (60.4 per cent), whereas 46 of the 55 control mice had given rise to tumors (83.3 per cent) in the same time. Thus during the later stages of the experiment the L.M.N. diet appeared to delay the induction of tumors by methylcholanthrene. When a supplement of fresh raw (unpasteurized) milk was given in place of the water that the controls received, there was a closer approach to the situation in respect to induced tumors than was manifested by the controls. On the other hand, when xanthine plus riboflavin was added to the raw milk, a greater deviation from the controls may have been observed. However, this should be verified upon larger groups of mice. It must be admitted that the very high levels of riboflavin and casein exerted little or no protective action against the tumors induced with the 1 mgm. dose of methylcholanthrene. The survival times of the mice after the actively growing tumors were recorded are given in Table I.

#### DISCUSSION

The 1 mgm. dose of methylcholanthrene injected subcutaneously is relatively high, and may have been so large as completely to overshadow any possible dietary influence. However, a number of other investigators have fed liver in attempts to influence tar, methylcholanthrene, or benzpyrene carcinogenesis with similar results. Watson (25) found that tar cancer in mice was slightly accelerated by feeding liver, and Bonser and Wainman (5, 6) that feeding fresh liver to benzpyrene-painted mice caused a slight acceleration of papilloma formation, but a delay in the development of malignant tumors. Although their experiment involved painting with a less potent carcinogen than methylcholanthrene, their results were in agreement with those reported here. Baumann and Rusch (3, 4) have shown, also, that feeding liver has little or no influence on carcinogenesis induced by ultraviolet light or by the local application of purified hydrocarbons. The possibility of demonstrating an influence of liver, milk, or riboflavin feeding by repeating these experiments with lower doses of methylcholanthrene therefore seems remote.

The investigators who produced hepatomas in rats by feeding *p*-dimethylaminoazobenzene (15, 18) were able to prevent this type of cancer by adding large supplements of liver (5 per cent) or yeast (15 per cent) to the basic rice diet. A year or two later Mori and Nakahara (17) found that whereas feeding liver prevented *p*-dimethylaminoazobenzene hepatomas, it did

not inhibit methylcholanthrene or benzpyrene tumors. Sugiura and Rhoads (23) began an investigation to ascertain the nature of the substances in the rice bran extracts, yeast, or beef liver that were responsible for the prevention of *p*-dimethylaminoazobenzene hepatomas. They found that casein alone or riboflavin alone exerted no protective action, but that partial protection was afforded by a supplement of a small amount of riboflavin fed with casein (14). Miller and others (16) studied this same problem, employing diets in which the adequacy of vitamins and proteins was more easily controlled. They concluded that nutritionally adequate diets offered at least partial protection against hepatoma formation, and stated the now generally accepted idea that protective supplements are usually rich in both protein and vitamin B complex, particularly riboflavin, and that nonprotective diets are deficient in at least one of these factors. According to the work of Axelrod and Elvehjem (1), mentioned earlier, a deficiency of either protein or riboflavin would result in a lowered xanthine (oxidase) dehydrogenase activity of the liver.

Thus it appears that *p*-dimethylaminoazobenzene induces hepatomas only in livers with low xanthine dehydrogenase resulting from a deficient or near-deficient diet, for when adequate or excess amounts of liver, yeast, or milk (24), xanthine dehydrogenase precursors, are fed along with *p*-dimethylaminoazobenzene, its carcinogenic action is diminished or noneffective. Carcinogenesis by substances like methylcholanthrene, on the other hand, is not dependent on a deficiency state, and little or no protection is afforded by a diet containing adequate or excess amounts of xanthine dehydrogenase precursors (high casein riboflavin) in the form of liver, milk, and riboflavin supplements.

#### SUMMARY

A diet of liver supplemented by combinations of raw, unpasteurized milk, riboflavin, and xanthine had little or no influence on the latent period or growth rate of tumors induced in C3H mice by the subcutaneous injection of 1 mgm. doses of methylcholanthrene. In general, the diet supplements stimulated tumors at first and later seemed to have a slight inhibitory effect. This effect was most pronounced in the group of mice receiving raw milk and riboflavin in addition to the basic dried liver, milk, Nurishmix diet. In any case, the effect of diet was so slight that its significance was questionable. This is in contrast to the remarkable inhibition of the carcinogenic action of *p*-dimethylaminoazobenzene observed by other investigators when adequate amounts of yeast, liver, or milk are added to the diet.

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