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Effect of Vitamin B_{12}-Deficiency in Colon Carcinogenesis (40776)

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Vitamin B_{12} is essential for normal growth and proliferation of cells (1). Biochemically, the basic roles of vitamin B_{12} are (a) as a coenzyme for methyl transferase, particularly to form methionine from homocysteine (2) and (b) as a coenzyme in the methyl malonyl mutase reaction (3). However, vitamin B_{12} may play a role in the methylation of some macromolecules, such as hypermethylation of bases, and thus could alter the normal genetic pathway to that of malignant tissues as demonstrated by hypermethylation of purines with dimethylnitrosamine (4).

Azoxymethane, a precursor of methylazoxymethanol (5), may express itself as a carcinogen by methylating some of these molecules. Vitamin B_{12} may play a role in this methylation process. Thus, it was of interest to test whether a deficiency of vitamin B_{12} would decrease the carcinogenic potency of azoxymethane.

Materials and methods. A commercial vitamin B_{12}-deficient diet was purchased from General Biochemical Inc., Canoga Falls, Ohio. Vitamin B_{12}-adequate diet was made up with 100 μg of vitamin B_{12} being added to a kilogram of deficient diet. Ground Wayne Lab Blox (Wayne lab meal) was used as control diet.

Weanling male F344/cr strain rats were obtained through the courtesy of Samuel Poiley, NCI. After a week on vitamin B_{12}-deficient diet supplemented with 50 μg of vitamin B_{12} per kilogram of the deficient diet, the rats were separated into various groups: vitamin B_{12}-deficient diet, vitamin B_{12}-deficient diet supplemented with B_{12} (100 μg/kg), Wayne lab meal, and our semisynthetic diet (Diet A; 6). Ten rats from each of the dietary groups were injected subcutaneously with 0.2 mmol (14.8 mg in saline) azoxymethane (AOM) per kilogram body weight once per week for 10 weeks, with controls receiving saline alone. The rats were then allowed to continue for another 10 weeks on their respective diets. All rats were examined thoroughly and weighed weekly. The animals were sacrificed 20 weeks following the first injection. At necropsy hematocrits and blood smears were taken from the tail vein. The liver and kidneys were removed, weighed, and fixed for histopathology. Other organs were fixed in a modified Telleysniczky's alcohol, acetic acid, and formalin fixative. The stomach and intestines, including small, large, caecum, rectum, and anus were carefully removed, placed on filter paper and slit open all the way in order to examine the interior of the complete gastrointestinal tract. The location of every lesion with respect to the distance from the anus for colon and from the stomach for small intestine, and also the size of each lesion were measured and recorded. Any lesions noted as well as sections of other organs were stained routinely with hematoxylin and eosin.

Results. The body weight gains of the vitamin B_{12}-supplemented and Wayne lab meal group were similar, demonstrating that vitamin B_{12} seemed to be the only essential dietary component for normal growth lacking in the deficient diet. The vitamin B_{12}-deficient diet did not affect the growth until about the eighth week on the diet. All the rats injected with azoxymethane did not grow as well as their dietary control counterparts. However, the stress of both carcinogen and vitamin-deficient diet had a profound effect on the growth of the rats in that group (Fig. 1).

The livers and kidneys of rats on the test diets with or without added vitamin B_{12} were much larger than those of rats on the commercial diet (Table I). The hematocrits of the rats in the diet control groups and

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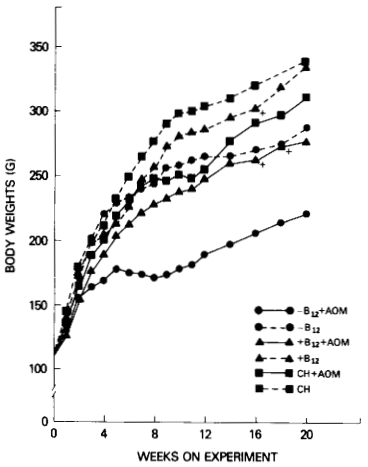

FIG. 1. Growth curves of rats on vitamin B_{12}-deficient diets. Growth curves are of rats on vitamin B_{12}-deficient diet, vitamin B_{12}-deficient diet supplemented with 100 μg vitamin B_{12} per kilogram diet and Wayne Lab Blox. Rats receiving 0.20 mmol azoxymethane per kilogram rat once a week for 10 weeks are connected with solid lines (——) and dietary controls with dotted lines (– – –).

vitamin B_{12}-deficient diet group were within the normal range, whereas those treated with AOM had lowered hematocrits corresponding to the presence of tumors. Many of the rats with colon tumors had marked anal bleeding.

The tumor incidences in all the rats receiving the carcinogen are shown in Table II. All of the vitamin B_{12}-supplemented group had both small intestinal (mainly duodenal) and colon tumors, averaging 1.2 and 1.5 tumors per rat, respectively. Of the three rats on the vitamin deficient diet with intestinal tumors, only one rat had both small and large intestinal tumors, while the others had either colon or duodenal tumors. The vitamin B_{12}-deficient diet group started to gain weight at a slower rate from the second week. The two rats that developed tumors also did not grow as rapidly as the vitamin B_{12}-supplemented group, which gained as much as those on Wayne lab meal. As anticipated, not all of the rats on the Wayne lab meal developed colon tumors under this dosage for this duration of administration of carcinogen (7). All tumors in the colon and small intestines were diagnosed as adenomas or adenocarcinomas (7, 8). Some of the lesions which appeared like tumors grossly were swollen Peyer's patches, and thereby, were not included.

Many of the rats had ear duct tumors which did not seem to affect the colon tumorigenesis except for inhibiting them from eating normally. Other histopathological lesions were observed in the enlarged livers and kidneys of the rats on the test diet with or without vitamin B_{12}-supplementation. The enlargement of the livers was due to the diet and was the result of both hyperplasia and hypertrophy of the hepatic cells. Few of the livers of rats receiving AOM had areas of hyperplasia as seen in rats treated with hepatocarcinogens. The kidneys of these rats were also enlarged and swollen resembling the nephrotic syndrome of older rats.

Discussion. Vitamin B_{12} is an important cofactor in the methyl transfer from 5-methyltetrahydrofolate to homocysteine to form methionine (2). Vitamin B_{12} may possibly play a role in methylation of mac-

TABLE I. BODY AND ORGAN WEIGHTS OF F344 RATS ON VITAMIN B_{12}-DEFICIENT RATS

Group	No.	Body wt. (g)	Liver (g/100 g rat)	Kidney (g/100 g rat)	HcT (%)
$-B_{12}$ + AOM	(10)	223 ± 5[a]	5.03 ± .12	1.21 ± .02	46.3 ± 0.8
$-B_{12}$	(5)	295 ± 7	4.33 ± .26	1.41 ± .01	42.1 ± 0.8
$+B_{12}$ + AOM	(8)	275 ± 5	4.10 ± .28	1.11 ± .04	39.4 ± 1.9
$+B_{12}$	(5)	330 ± 16	4.52 ± .67	1.10 ± .10	47.0 ± 0.3
Chow + AOM	(6)	309 ± 9	2.90 ± .04	.65 ± .02	35.6 ± 3.4
Chow	(5)	342 ± 12	3.08 ± .04	.64 ± .01	42.8 ± 0.7
Special[b] + AOM	(10)	299 ± 10	2.64 ± .06	.59 ± .02	38.7 ± 3.2
Special	(5)	327 ± 10	2.82 ± .05	.60 ± .01	45.9 ± 1.5

[a] Average weight ± SEM.
[b] Special diet—a semipurified diet.

TABLE II. TUMOR INCIDENCES IN RATS RECEIVING AZOXYMETHANE

		Tumors				
		Ear duct	Small intestine		Colon	
Diet	No. of rats	No. of rats	No. of rats	No. of tumors (per rat)	No. of rats	No. of tumors (per rat)
$-B_{12}$	(10)	3	2	2 (0.2)	2	4 (0.4)
$+B_{12}$	(8)	3	8	10 (1.2)	8	12 (1.5)
Chow	(6)	1	6	8 (1.3)	4	5 (0.8)
Special	(10)	2	10	18 (1.8)	10	24 (2.4)

romolecules involved in carcinogenesis. Thus, a lack of vitamin B_{12} may slow the carcinogenic process which involves methylation of macromolecules as observed with dimethylnitrosamine and dimethylhydrazine (9–13).

Vitamin B_{12} deficiency has been shown to be somewhat effective in preventing liver carcinogenesis from dimethylaminoazobenzene (14, 15). Since vitamin B_{12} is important in liver metabolism, the lack of vitamin B_{12} may have affected the liver cells themselves. In the case of the colon carcinogens, 1,2-dimethylhydrazine, and AOM, the liver plays a role in the metabolism of these carcinogens but is not the main target organ. To avoid the metabolic activation steps required with 1,2-dimethylhydrazine, AOM was considered a more suitable carcinogen.

The failure of the development of colon tumor in vitamin B_{12}-deficient rats may be due to decrease in methylation of macromolecules involved in carcinogenesis. Magee *et al.* (9–11, 16) suggested that the methylation of macromolecules was important in dimethylnitrosamine carcinogenesis. Two of the ten rats on the vitamin B_{12}-deficient diet did develop colon tumors. They may have done so possibly because they may not have been completely deficient of vitamin B_{12}, that is, they may have had sufficient storage of vitamin B_{12} to affect the methylation during early carcinogenesis. For this reason, the deficient diet was supplemented with a low concentration of vitamin B_{12} (50 μg/kg diet) rather than the deficient diet alone for dietary and environmental adaptation purpose before the start of the experiment. The only noticeable physiologic difference between the two and the other rats in their group was the lowered hematocrit. However, the lowered hematocrit is a common finding in all rats with colon adenocarcinoma. The vitamin B_{12}-deficient diet did not have any effect on the growth of the rats until about the eighth week. The stress of both vitamin deficiency and AOM injection seemed to affect the growth rate of the doubly stressed rats more than those singly stressed.

Only six rats were used as Wayne lab meal-positive controls since these controls in many of our earlier studies were always positive with 80–100% having colon tumors under these same conditions (7). In this experiment with the level of 0.2 mmol AOM per kilogram rat only four of six rats had colon tumors. The rats on Wayne lab meal seem to require a slightly higher dose of carcinogen to always produce 100% tumorigenesis. All the rats fed the experimental diet with 100 ppb vitamin B_{12} developed colon tumors when administered AOM, similar to those rats on our semisynthetic diet (6). Thus there seems to be a modifying factor in the Wayne lab meal as yet undetermined.

Summary. The effect of vitamin B_{12} deficiency on intestinal carcinogenesis with azoxymethane was studied in male F344 rats. Upon weekly injection of 0.2 mmol/kg body wt of azoxymethane, most of the rats on vitamin B_{12}-deficient diet failed to produce small intestinal or colon tumors, whereas, all of the rats on vitamin B_{12}-deficient diet supplemented with 100 μg/kg vitamin B_{12} developed adenoma or adenocarcinoma of duodenum or colon. The vitamin B_{12}-adequate diet was more effective in producing colon tumors in F344

rats than mixed commercial diet. It appears that vitamin B_{12} is necessary for intestinal carcinogenesis with azoxymethane.

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