Influence of Pantothenic Acid Deficiency on the Viability and Growth of a Rat Fibrosarcoma*

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INTRODUCTION

It has been reported by Morris *et al.* (6, 7) that dietary pantothenic acid deficiency retarded the growth of spontaneous mammary tumors in mice (C3H strain). This result is in contrast to the findings of Bischoff *et al.* (2) who found no retardation of growth of Sarcoma 180 transplanted into Marsh-Buffalo mice on a similar pantothenic acid-deficient diet. Although different tumors and different strains of mice were used, the latter authors suggested that their negative results might be owing to failure to deplete the endogenous stores of pantothenic acid.

The present experiment was designed to test the effect on the growth of a rat fibrosarcoma of a diet deficient in pantothenic acid, as well as that of an anti-metabolite of this vitamin, ω-methylpantothenic acid (3).

EXPERIMENTAL

Thirty-six adult female rats of the Long-Evans strain were divided into four groups. Group I, containing ten control animals, was maintained on a modified Anderson and Smith (1) diet (Diet I, Table 1), and Group II, composed of nine control animals, was fed a modified Ershoff (5) diet (Diet II, Table 1). Groups III and IV, containing nine and eight experimental rats, respectively, were maintained on the modified Ershoff diet devoid of pantothenic acid. Group IV received, in addition to the deficient diet, injections of ω-methylpantothenic acid as described below.

After 1 week on their respective diets, the rats received inoculations of tumor tissue. A piece of fibrosarcoma tissue (4), approximating a volume of 30 c. mm., was implanted subcutaneously by trocar into each of two axillary and two inguinal ventral sites of each rat.

Just prior to i mplantation of the tumor tissue, 200 mg. of ω-methylpantothenic acid (1 ml. of aqueous solution sterilized by passage through a Mandler filter) was injected subcutaneously into each rat in Group IV. The dose of the analog was reduced to 100 mg. on the second day and to 50 mg. on the third day after implantation. Injection at this dosage level was continued daily for the following 6 days. Since a rapid and continuous loss in body weight was observed, similar injections were given only on alternate days for the ensuing 2½ weeks. Normal saline was administered to the animals in Group III in an identical manner.

The growth of the tumors at each site was followed by estimating the maximum tumor area from caliper measurements. An initial increase followed by a 10 per cent or greater decrease in size or the total disappearance of the tumor was deemed regression.

TABLE 1

COMPOSITION OF THE DIETS

Diet I		Diet II	
Constituent	Per cent	Constituent	Per cent
Commercial casein	24	Vitamin-test casein*	22.0
Whole milk powder	24	Sucrose	73.2
Wheat germ + 20 per cent middlings	20	Salt mixture†	4.5
Brewer's yeast	3	Corn oil supplement‡	0.75
Swiftning§	29	L-cystine	0.3
		Choline chloride‖	0.1

* Hot-alcohol extracted casein (General Biochemicals, Inc.) was employed. According to the manufacturer's specification, the maximum limit of pantothenic acid present was 0.45 μg. per gram. Nelson *et al.* (8) reported that this brand of casein contained 1.3 μg. of pantothenic acid per gram, primarily in the bound form.

† Salt mixture No. 1 of Sure (10).

‡ Constituents: Vitamin A, 20,000 USP units; Vitamin D, 2,000 USP units; α-tocopherol (Merck), 50 mg.; 2-methyl-1,4-naphthoquinone (Menadione), 1 mg.; corn oil (Mazola), 9.85 gm.

§ Swift & Co., Shortening made from animal and vegetable fats.

‖ Other vitamins added, in mg/kg of above diet: thiamin, 5; riboflavin, 10; pyridoxine HCl, 5; niacinamide, 100; inositol, 400; p-aminobenzoic acid 150; biotin, 0.2; and calcium pantothenate, 20.

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RESULTS AND DISCUSSION

It may be seen from the results given in Table 2 that there was no significant difference in the percentage of successful implantations (viability) of the tumor in the control and experimental groups. The number of established tumors which subsequently regressed, however, was greater in the experimental animals than in the controls. The percentage of tumors which regressed was not increased through use of the inhibitor.

An apparent retardation of tumor growth is indicated by the areas given in Table 2. The difference between the two control groups is relatively small, while that between the control and experimental groups is marked and roughly proportional to the degree of (dietary and induced) pantothenic acid deficiency. The similarity between the control groups is emphasized by the average terminal tumor weight data. While direct comparison between terminal tumor weights of the control and experimental groups is vitiated by the difference in time, the relatively low terminal tumor weight in Group IV substantiates the apparent retardation of tumor growth under this regimen evinced by the data on tumor area.

TABLE 2

BASIC DATA

Group	No. rats	No. sites inoculated	No. takes (per cent)	No. tumors regressed (per cent)	No. nonregressive tumors	Av. tumor size, Days after implantation: 17 (cm.²)	22 (cm.²)	27 (cm.²)	30 (cm.²)	34 (cm.²)	38 (cm.²)	Av. terminal tumor wt. (gm.)	Body weight data*: Initial (gm.)	Final (gm.)	Diff. (gm.)
I. Anderson and Smith diet (control)	10	40	92	8.1	34	1.5	2.7	4.2				5.8	276	255	−21
II. Ershoff diet (control)	9	36	94	8.8	31	1.3	2.2	3.4	4.1			5.7	277	259	−18
III. Pantothenic acid-free diet (exper.)	9	36	92	27	24	0.18	0.78	1.8	2.4	3.5	5.1	7.7	253	220	−33
IV. Pantothenic acid-free diet plus inhibitor (exper.)†	8	32	100	28	23	0.25	0.92	1.3	1.8	2.5	3.2	4.3	263	194	−69

* Total weight minus tumor weight.

† ω-methylpantothenic acid.

While all the groups lost weight during the experiment, the only significantly different value was that for Group IV, in which the weight loss was approximately 3 times the average of the other three groups.

From the data in Table 3 it is apparent that the average rate of tumor growth, based on terminal tumor weights, was essentially the same for Groups I–III and significantly lower for Group IV. The reduced rate in this group is thought to reflect the markedly higher rate of body weight loss. This belief is supported by the similar ratios between average tumor weight and average body weight in Groups I–IV. An apparent inverse relationship between rate of tumor growth and rate of body weight loss is indicated by the curve given in Chart 1.

Schrek (9) has shown that the geometric mean of the diameters of a tumor (Walker rat tumor and Flexner-Jobling rat carcinoma) plotted against time yields a straight line, the slope of which represents the growth rate and whose intercept with the time-axis is a fair estimate of the "lag-period" of the growth of the tumor. The data of the present experiment have been treated in this manner (mean diameter = $\sqrt{d_1 \cdot d_2}$), and the resultant curves for nonregressive tumors are given in Charts 2 and 3. It may be seen in Chart 2 that the apparent retardation in growth rate shown by the areas tabulated in Table 2 results more from a difference in lag-period than from a marked difference in growth rate per se. The average latent period for the control groups was 2 days, while that of the experimental groups was 12 days. The superimposition of these curves (Chart 3), without regard for the time values, emphasizes the lack of significant differences in the growth rates for Groups I–III. The break in the curve for Group IV and the decreased slope of the second portion of the curve may reflect tumor-growth retardation. These results would lead to the conclusion that, with the possible exception of the inhibitor group (Group IV), there is no significant differ-

ence in the rates of tumor growth per se in the various groups but that there is definitely a greater "lag-period" in the initiation of active growth when a deficiency of pantothenic acid exists. The retardation in the rate of tumor growth in Group IV may be related, however, to the significant difference in body weight loss. Growth

tissue in a series of seven uniform aliquots of a tumor suspension was 22.1 per cent, while the deviations for two sets of five trocar-implant samples were 5.0 and 9.6 per cent. Routine tests for bacterial infection of transplant material consisted in culturing sample pieces of tissue in fluid thioglycollate and in nutrient broth. Suspect

TABLE 3

DERIVED TERMINAL DATA ON NONREGRESSIVE TUMORS

Growth	Av. rate tumor growth (gm/day)	Av. rate body weight loss (gm/day)	Av. tumor weight / Av. body weight*	Av. tumor weight (gm.) / Av. tumor size (cm.³)
I. Anderson and Smith (control)	0.21	0.78	0.023	1.4
II. Ershoff (control)	0.19	0.60	0.022	1.4
III. Pantothenic acid-free diet	0.20	0.87	0.035	1.5
IV. Pantothenic acid-free plus inhibitor†	0.11	1.8	0.022	1.3

* Average Standard Deviation = 0.017.
† ω-methylpantothenic acid.

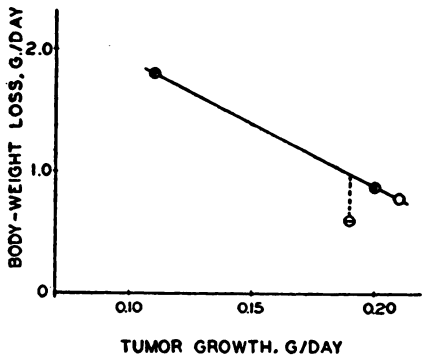

CHART 1.—Curve relating body weight loss and nonregressive tumor growth of female Long-Evans rats on various diets.

The notations are as follows:
O—Control Group I, modified Anderson and Smith diet.
⊖—Control Group II, modified Ershoff diet.
⊙—Experimental Group III, Pantothenic acid-deficient diet.
⊕—Experimental Group IV, Pantothenic acid-deficient diet plus ω-methylpantothenic acid injection.

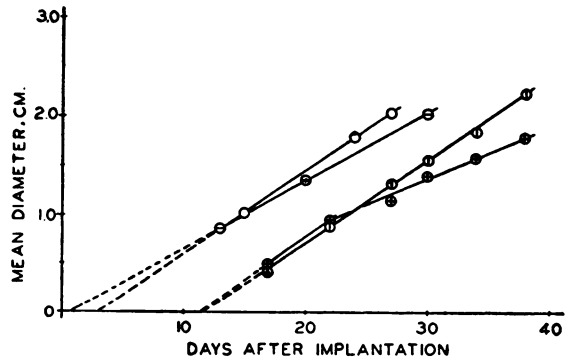

CHART 2.—Curves relating mean diameter of nonregressive tumors and time.

The notations are as follows:
O—Control Group I, modified Anderson and Smith diet.
⊖—Control Group II, modified Ershoff diet.
⊙—Experimental Group III, pantothenic acid-free diet.
⊕—Experimental Group IV, pantothenic acid-free diet plus injections of ω-methylpantothenic acid.

curves for the regressive tumors are given in Chart 4.

The trocar method of implantation has been adopted as a standard procedure in this laboratory, since preliminary studies had shown that this method yielded more consistent results than the injection of a suspension of homogenized tissue. This result was ascribed to variations in the amount of tissue introduced by the latter method and to the possibility of infecting the tumor tissue. In a comparative study of the two methods, the deviation from the mean dry weight of injectable

cultures were then plated out on agar. Only once in five successive transplantations of trocar-implanted tissue was bacterial growth found, and it was not carried through by the tissue into subsequent transplantations. Infection of a pyogenic nature was encountered more often in the tissue inoculated in the form of a suspension and was eliminated, finally, by resorting to the trocar method. The tumor tissue implanted in the present experiment was shown to be bacteriologically sterile by the above tests.

Utilization of caliper measurements to follow the growth of tumors has been employed by many

authors. Schrek (9) has shown that the geometric mean of three diameters of a tumor, *in situ*, could be determined with an average error of 3.8 per cent, and that the average errors of the calculated tumor volumes and weights, based on the mean diameter, were within 10–11 per cent of the experimentally determined values. Voegtlin and Thompson (11) reported a uniform relationship between area (product of two diameters) and weight of transplants of Hepatoma 31. In the present experiment, the ratios of terminal tumor weight to terminal tumor size (area), given in Table 3, were all within 7.1 per cent of the mean value.

Ruffled hair, hyperirritability, exudate on the face and whiskers, blood-shot eyes, and other symptoms reported by Woolley (12) and others (3) as characteristic of pantothenic acid deficiency, were observed to a marked degree in Group IV and to some extent in Group III. That the retardation of the growth of the tumors may merely reflect the total systemic effect of pantothenic acid deficiency is indicated by the lack of significant differences between the ratios of terminal tumor weights to terminal body weights. This interpretation is in agreement with the conclusions of Morris *et al.* (7) who stated that ". . . retardation of tumor growth by a diet deficient in pantothenic acid results simultaneously in such severe interference in the host's nutrition that the procedure offers no practical application in adjunct tumor therapy."

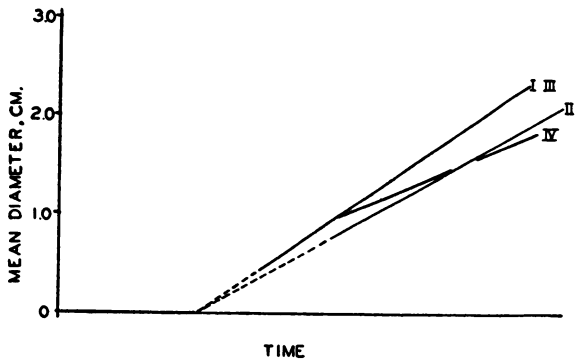

CHART 3.—Curves relating mean diameter of nonregressive tumors and time.

The Roman numerals refer to the group number.

The results of the present authors, as well as those of Morris *et al.* (6, 7), differ from those of Bischoff *et al.* (2). It is interesting to note that Morris *et al.* and Bischoff *et al.* also obtained diametrically opposite results with pyridoxine deficiencies. This lack of similarity might be ascribed, tentatively, to differences in the vitamin requirements of the types or strains of animals or tumors employed.

CONCLUSIONS

It is concluded from these experiments that pantothenic acid deficiency for 1 week prior to inoculation of a fibrosarcoma in rats had no effect upon the viability of the tumor implants. Continued deficiency caused an apparent retardation of growth of the tumors and increased the percentage of regressions. The retardation of growth was shown to derive from an increased "latent" period rather than from a decreased growth rate per se. The injection of a pantothenic acid antimetabolite, ω-methylpantothenic acid, into animals on a pantothenic acid-deficient diet did not further increase the percentage of regression or the length of the "latent" period. The decreased tumor growth of the animals treated with the inhibitor apparently relates directly to the significantly greater body weight loss under this regimen. The retardation of tumor growth by pantothenic acid deficiency appears merely to reflect the total systemic effect of such a deficiency and does not indicate a specific effect on tumor tissue.

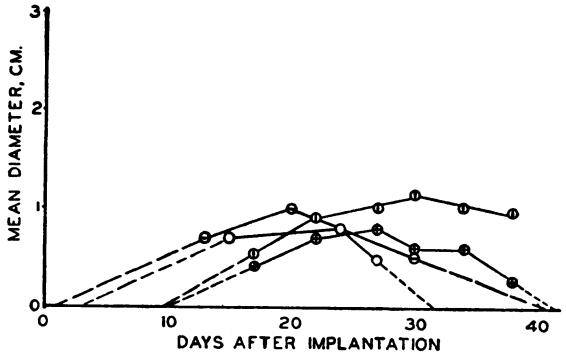

CHART 4.—Curves relating mean diameter of regressive tumors and time.

The notations are as follows:

○—Control Group I, modified Anderson and Smith diet.

⊖—Control Group II, modified Ershoff diet.

⊙—Experimental Group III, pantothenic acid-free diet.

⊕—Experimental Group IV, pantothenic acid-free diet plus ω-methylpantothenic acid injection.

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