Nucleic Acids and Their Components in Tumor-Bearing Mice during Growth and Regression of Tumors.* (19719)

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In recent years, a great deal of attention has been directed to the part played by nucleic acids in neoplastic growth. Several investigators(1,3,5,8,13,14) have presented evidence indicating a high nucleic acid content for tumor tissues. It has been shown recently in this laboratory(2,7,9) that there is an increase in the nucleic acid content of the liver, kidney, and lung of mice bearing a transplantable sarcoma.

Bischoff *et al.*(21) reported that a synthetic diet containing vitamins of the B complex other than B_6 produced a marked decrease in the growth rate of sarcoma 180 in Marsh-Buffalo mice, and addition of vit. B_6 increased it again. On the other hand, Morris(22) found that the growth of spontaneous mammary adenocarcinoma in strain C3H mice was decreased by extreme deficiency of pantothenic acid and riboflavin produced rapidly during a short period, and was not affected by extreme deficiency of pyridoxine.

Stoerk(15) reported the regression of lymphosarcoma implants in mice which were receiving desoxypyridoxine on a pyridoxine deficient diet. This experiment was repeated by us with a view of making a comparative study of the nucleic acids and their purine constituents in mouse tissues during tumor regression. Because of the pronounced changes in desoxyribose nucleic acid (DNA) which were found during tumor regression in the spleen, the pyrimidines were also determined in this tissue.

Experimental. Male mice of the C3H strain of approximately 5 weeks of age were employed in this experiment. A mouse lymphosarcoma,† 6-C3H-ED, was transplanted subcutaneously with a biopsy needle into the right pectoral region of the mice. The animals were placed on a stock diet (Purina Laboratory Chow) and were given ordinary tap water to drink.

The tumor implants were allowed to grow from 8 to 11 days at which time a number of animals were sacrificed, and their tissues separately pooled into groups of 4 animals each. The remaining animals were divided into 2 groups, experimentals and controls.

The experimental animals, designated "B_6 deficient," were placed on a diet deficient in vit. B_6. The composition of the diet was as follows: Vitamin-free casein (Labco), 25 g; sucrose, 53 g; Crisco, 10 g; lard, 5 g; salts,‡ 5 g; Ruffex, 2 g; thiamine, 1 mg; riboflavin, 1 mg; choline, 150 mg; calcium pantothenate, 10 mg; vit. A,§ 6,750 I.U.; alpha-tocopherol, 4 mg; and vit. D,‖ 500 I.U. These animals also received desoxypyridoxine¶ in their drinking water, at a level of 300 μg per ml.

The controls, designated "B_6 sufficient," were placed on the same diet except that vit. B_6 was added at a level of 20 mg per 100 g of diet. They also received 300 μg of desoxypyridoxine per ml in their drinking water.

A number of animals from each group were sacrificed at different stages of tumor growth as shown in the figures. The experiments were carried out in 3 different sections and with a total of 126 mice. Ten mice on the vit. B_6 deficient diet and 6 mice on the vit. B_6 sufficient diet died during the course of the experiment. These mice were discarded.

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† Obtained from the Jackson Memorial Laboratory, Bar Harbor, Me.

‡ Osborne and Mendel.

§ Given in the form of a concentrate (1 000 000 I.U./g) supplied by Nopco Chemical Co., Harrison, N. J.

‖ Given in the form of Drisdol (Winthrop-Stearns Inc., New York).

¶ Kindly supplied by Merck and Co., Rahway, N. J. through the courtesy of Dr. Karl Folkers.

TABLE I. Tumor Development in Mice Receiving Desoxypyridoxine on a Vit. B_6 Deficient Diet and on a Vit. B_6 Sufficient Diet.

a. Treatment—desoxypyridoxine plus a vit. B_6 deficient diet

Before treatment				After treatment			
No. of animals	Body wt (g)	Tumor diam. (cm) Avg	Range	No. days on treatment	Body wt (g)	Tumor diam. (cm) Avg	Range
4	20	1.6	(1.3–1.7)	7	16	1.3	(1.2–1.3)
6	22	3	(2.6–3.2)	7	17	.6	(.4– .9)
5	23	2.2	(2 –2.5)	8	19	.7	(0 –1)
4	19	1.5	(1.4–1.7)	9	15	.3	(0 – .7)
3	23	2.1	(1.9–2.2)	10	19	.4	(0 –1)

b. Treatment—desoxypyridoxine plus a vit. B_6 sufficient diet

Before treatment				After treatment			
No. of animals	Body wt (g)	Tumor diam. (cm) Avg	Range	No. days on treatment	Body wt (g)	Tumor diam. (cm) Avg	Range
6	19	1.5	(1.3–1.7)	7	26	3.6	(3.4–4)
8	21	3	(2.9–3.1)	7	26	3.9	(3.6–4.6)
5	22	2.1	(1.9–2.3)	8	29	4.3	(3.8–4.7)
6	18	1.5	(1.2–1.6)	9	25	4.3	(3.8–4.5)
4	24	2.2	(2 –2.5)	10	27	4.1	(3.7–4.7)

All animals were sacrificed by decapitation and the tissues upon removal frozen immediately with dry ice. The tissues (liver, kidney, lung, spleen, and tumor) were separately pooled into groups of 4 or 5 and homogenized in a glass homogenizer using distilled water at 0°C. All homogenates were approximately 10-15% suspensions.

Aliquots of 2 ml were removed for nucleic acid analysis, pyrimidine analysis, and dry weight determinations. For the latter, all samples were dried at 105-110°C to constant weight. The nucleic acids were extracted by the trichloroacetic acid method of Schneider (12). One ml of the extract was utilized for the estimation of ribose nucleic acid (RNA) according to the method of von Euler and Hahn(4) and 0.5 ml for the estimation of desoxyribose nucleic acid (DNA) by Stumpf's (16) method. The nucleic acids in the remaining extract were hydrolyzed by heating with 1 N H_2SO_4 in a boiling water bath for 2 hours, under which conditions we have found that the purines (adenine and guanine) are completely liberated. Adenine was estimated in the hydrolysate by Woodhouse's(18) method and guanine by the method of Hitchings(6).

The pyrimidines (uracil, cytosine, and thymine) in the spleen were determined by paper chromatography according to the procedure of Vischer and Chargaff(17) with the modifications used by Cerecedo *et al.*(2). Wyatt's (19) solvent was used to develop the chromatograms, making possible the complete separation of the pyrimidines with one solvent.

Analyses were also performed on normal animals covering the entire experimental period.

Results. In Table I are presented data on the average tumor diameter before and after the treatment specified, the ranges of the tumor diameters, and body weights. It will be seen that the tumor grows rapidly in mice receiving desoxypyridoxine on a vit. B_6 sufficient diet. On the other hand, the tumor regresses in mice receiving desoxypyridoxine on a vit. B_6 deficient diet. The regression is as great as 100% in certain cases after 8 days treatment with desoxypyridoxine on a vit. B_6 deficient diet. These data clearly confirm Stoerk's(15) observation that lymphosarcoma implants regress in mice which receive a pyridoxine deficient diet and in addition desoxypyridoxine.

In one group of the vit. B_6 deficient animals the average loss in body weight was 5 g, whereas in the other 4 groups it was 4 g. On the other hand, the animals receiving desoxypyridoxine on a vit. B_6 sufficient diet gained weight. The vit. B_6 sufficient animals ate on the average 2.3 g of food per day while the vit. B_6 deficient animals ate 1.5 g of food per day. However, it must be remembered that

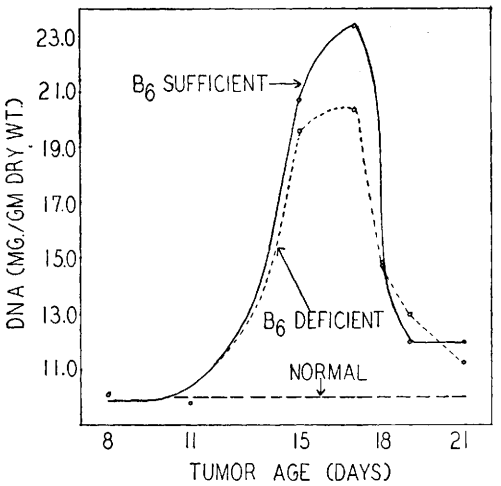

FIG. 1. DNA content of liver in tumor-bearing mice a) receiving vit. B_6, and b) deficient in vit. B_6.

caloric restriction on a tumor already established is relatively small. The major effect of caloric restriction is on the genesis or incidence of a tumor rather than on the growth of a tumor already firmly established(20).

The effects of desoxypyridoxine on a vit. B_6 deficient diet in the mouse seem to be specific. That is, it is not merely a general depression of body weight and a decrease in the rate of tumor growth. The data presented by Stoerk(15) and the data presented in Table I of this paper confirm this point.

In Fig. 1 the concentration of DNA in the liver (expressed in mg/g dry weight) against the tumor age in days has been plotted. The horizontal dotted line indicates the normal level of DNA in the liver. It may be seen that in mice on a vit. B_6 sufficient diet there is a 100% increase in DNA when the tumor is 15 days old. This increases further and is maximal when the tumor is 17 days old, but then decreases steadily and returns almost to normal at 21 days. The same trend is shown by animals on a vit. B_6 deficient diet except that the maximum at 17 days is not as high as that of the animals on a B_6 sufficient diet. RNA shows a similar trend (not shown in the figure), but again reaches a higher level in the vit. B_6 sufficient animals. In both the B_6 sufficient and deficient animals, the increases in RNA were not as great as those in DNA. This was evident from the RNA/DNA ratio which reached its lowest level when the tumor was 17 days old but then increased again returning almost to normal at 21 days. Adenine and guanine followed essentially the same trend as the nucleic acids. However, the increases in guanine were greater than those in adenine so that the ratio of guanine to adenine reached a maximum at 17 days of tumor growth.

Fig. 2 shows the concentration of RNA and DNA in the lung. The vit. B_6 sufficient animals again show an increase in DNA which is maximal at the 17th day of tumor growth. The deficient animals show a maximum in DNA at 15 days which then falls to normal at 21 days. RNA increases to a maximum at 17 days in the B_6 sufficient animals, and then drops almost to normal at 21 days. The B_6 deficient animals show the same trend but actually return to normal at 21 days. In each case, the nucleic acids reach a higher level in the vit. B_6 sufficient animals. Adenine and guanine show the same pattern as the nucleic acids (not shown on the figure).

In Fig. 3 the changes in RNA and DNA in the kidney are shown. The vit. B_6 sufficient animals show an increase in DNA which is maximal when the tumor is 17 days of age, and then drops. The deficient animals show

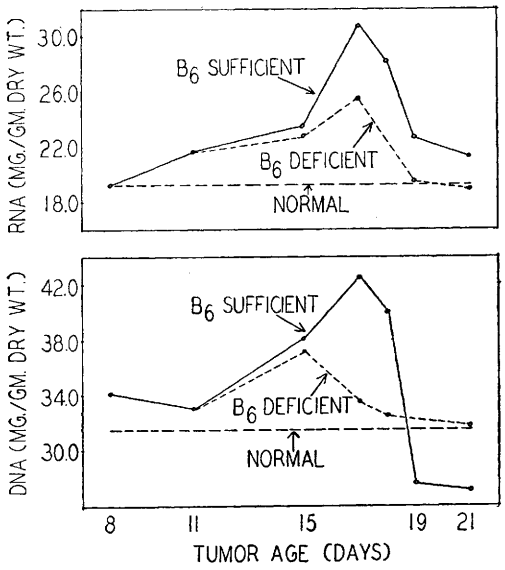

FIG. 2. DNA and RNA contents of lung in tumor-bearing mice a) receiving vit. B_6, and b) deficient in vit. B_6.

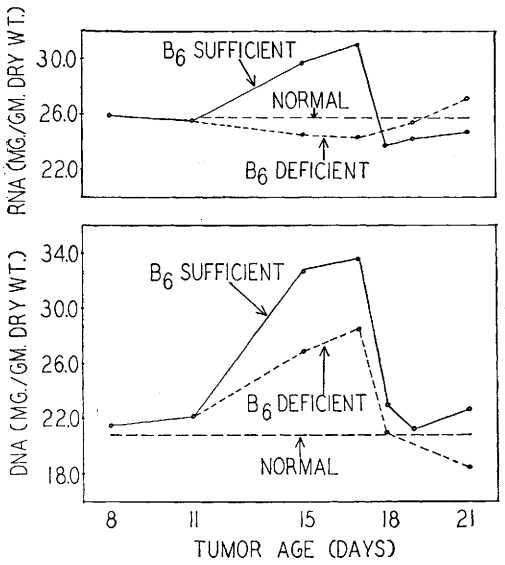

FIG. 3. DNA and RNA contents of kidney in tumor-bearing mice a) receiving vit. B_6, and b) deficient in vit. B_6.

a similar trend except that the increase in DNA is not as great. Adenine and guanine follow essentially the same pattern.

RNA does not rise above normal in the kidney of the deficient animals. On the other hand, it increases in the vit. B_6 sufficient animals to a maximum at 17 days and then falls. In addition to the liver and lung, therefore, the kidney presents further evidence that the nucleic acids reach a higher level in the vit. B_6 sufficient animals than in the deficient animals.

In the sarcoma (figure not shown), DNA and RNA reach a maximum at the 17th day of tumor growth. These are higher in the vit. B_6 sufficient mice. In each case, the rate of increase of DNA is greater than that of RNA up to the 17th day. Both DNA and RNA then decrease until at 21 days their values are comparable to the values at 11 days of tumor growth. Again, the rate of decrease of DNA is greater than that of RNA and at 21 days the ratio of RNA/DNA is comparable to that at 11 days.

In Fig. 4 the data for DNA and thymine in the spleen are given. Vit. B_6 sufficient animals show a maximum in DNA when the tumor is 17 days old. However, this value is close to normal. It then drops to a value approximately 20% below normal at 19 days.

The deficient mice show a quite different effect. In these animals, DNA drops quite sharply in the spleen. At 19 days this value is as low as 29.8 mg/g dry weight which is approximately 70% below normal. This is substantiated by a 70% drop in thymine from a value of 10.0 mg/g dry weight when the tumor is 11 days old to a value of 3.0 mg when the tumor is 19 days old. Cytosine, guanine, and adenine were found to exhibit a similar drop.

RNA remains close to normal in the spleen during tumor growth and regression, and this was verified by the fact that uracil remains fairly constant.

Discussion. The increase in nucleic acids in the various tissues during neoplastic growth is in agreement with previous results obtained in this laboratory. The data reported in the present paper indicate that there is an increase in the nucleic acids and their purine constituents in the liver, kidney, and lung during growth of a transplanted lymphosarcoma in C3H mice. These increases in the concentration of nucleic acids in the tissues of the host point to a stimulation of the nucleic acid

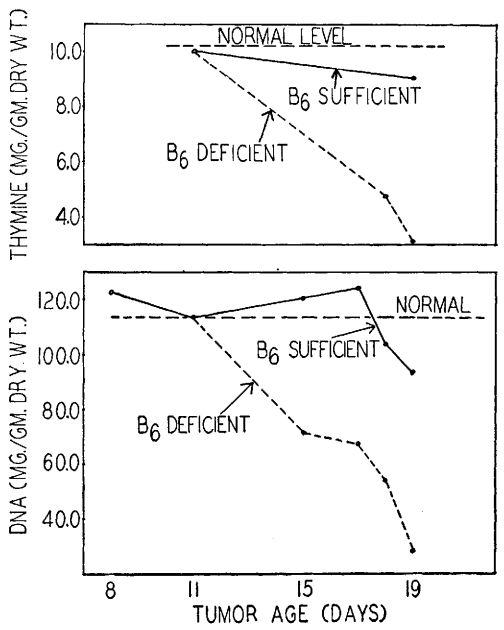

FIG. 4. DNA and thymine contents of spleen in tumor-bearing mice a) receiving vit. B_6, and b) deficient in vit. B_6.

metabolism during neoplastic growth.

It is of interest to point out that the DNA concentration at its highest level in the mouse lymphosarcoma (93.9 mg/g dry weight) is higher than that of Sarcoma 180 (49.1 mg/g dry weight)(7), and melanoma S-91 (61.7 mg/g dry weight)(2). This may be due to the high cellularity of the lymphosarcoma.

The fact that the nucleic acids reached a higher level in mice receiving desoxypyridoxine on a vit. B_6 sufficient diet than in mice receiving desoxypyridoxine on a vit. B_6 deficient diet suggests that desoxypyridoxine may act as an inhibitor in nucleic acid synthesis.

The pronounced drop in DNA in the spleen is very interesting. It has been suggested that pyridoxine is essential for the maintenance of the lymphocytes(10,11) of lymphatic tissue. Since the lymphocytes are rich in nucleic acids, it is possible to conceive that pyridoxine may play a fundamental part in nucleic acid synthesis possibly as a cofactor and thus maintain the normal lymphocyte level.

Summary. 1. The concentration of ribose nucleic acid (RNA), desoxyribose nucleic acid (DNA), adenine, and guanine of liver, lung, kidney, and spleen of normal C3H mice and those bearing a transplantable mouse lymphosarcoma at different stages of tumor development has been determined. The mice bearing the tumor were divided into 2 groups; one group received desoxypyridoxine on a vit. B_6 deficient diet, while the other received desoxypyridoxine on a diet rich in vit. B_6 to counteract the effect of the anti-vitamin. In addition to DNA, RNA, adenine, and guanine, the pyrimidines (uracil, cytosine, and thymine) were also determined in the spleen. 2. The data presented indicate that there is an increase in the nucleic acids and their purine constituents in the liver, kidney, and lung during the growth of the tumor. 3. The nucleic acids reached a higher level in mice receiving desoxypyridoxine on a vit. B_6 sufficient diet than in mice receiving desoxypyridoxine on a vit. B_6 deficient diet. 4. Mice receiving desoxypyridoxine on a diet high in vit. B_6 show a maximum value in DNA (which is close to normal) in the spleen on the 17th day of tumor growth which then drops to approximately 20% below normal at 19 days. In mice receiving desoxypyridoxine on a diet deficient in vit. B_6, DNA in the spleen drops quite sharply. At 19 days, this drop is over 70% below normal and this is followed by a similar drop in thymine, cytosine, guanine, and adenine. RNA does not vary to a significant extent in the spleen, and this is also true of uracil. 5. The changes in nucleic acids are followed generally by similar changes in adenine and guanine.

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