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Complete Regression of Lymphosarcoma Implants Following Temporary Induction of Riboflavin Deficiency in Mice.

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It has been found previously that lymphosarcoma implants show marked regression following the administration of a pyridoxine antagonist (desoxypyridoxine).[1] In recent experiments a number of other vitamin antagonists were examined for their possible effect upon lymphosarcoma (6C3H-CD) in mice of the C3H strain. It was found that the feeding of a diet low in riboflavin produced regression of established lymphosarcoma implants. This regression was enhanced by the administration of riboflavin antagonists (isoriboflavin,[2] galactoflavin.[3]).

C3H male mice 5-6 weeks of age were inoculated with lymphosarcoma (6C3H-ED)* fragments under sterile precautions. The tumor transplants were placed under the skin of the lower back. The tumors were then permitted to grow for 6 to 14 days. During this time the animals were maintained on a complete diet of natural foods. They were then divided into experimental groups and subjected to treatment as indicated in the table. At the end of the feeding periods the animals were transferred back to the stock diet. The findings summarized in the table show that in 51 control animals on a stock diet (Group I), the tumors attained an average size of 9.2 cm^3 within 20 days. After 30 days there were no survivors in this group. Mice fed for 14 days (group II) and for 26 days (group III) a diet deficient in vitamin B_2 showed marked to complete regression of the established implants within 10 days. Thirty per cent of the mice in group II and 37% in group III survived for more than 60 days, a period after which no recurrences were ever observed up to 200 days. Amounts of riboflavin required for maintenance in C3H mice, when fed to lymphosarcoma bearing animals (Group IV), were not sufficient to permit growth of the implants at the usual rate. However the rate of tumor growth did not significantly differ from that in the controls (Group I) when 8 μg (Group IX) or 10 μg (Group V) were given daily to the animals. Tumors that were permitted to grow to a larger size (Group VIII) regressed rapidly when a riboflavin antagonist was administered. The feeding of a riboflavin analogue Group VI and VII) was rendered ineffective when 100 μg of vitamin B_2 were given simultaneously. Animals surviving for more than 60 days, when reinoculated with lymphosarcoma tissue, failed to "take" the second implant. The administration of other vitamin antagonists (pyrithiamine, 3-acetyl pyridine and pteroyl aspartic acid) had no significant effect upon the lymphosarcoma implants.

Regression of lymphosarcoma transplants, produced by the administration of a pyridoxine antagonist (desoxypyridoxine),[1] occurred more rapidly than the regression observed in riboflavin deficiency. However refeeding of vitamin B_6 deficient animals with pyridoxine was followed by recurrence of the tumors. In mice in which tumor regression had been induced by transitory vitamin B_2 deficiency, the tumor did not recur when riboflavin was fed. Moreover in these mice even reinoculation was ineffective. This suggests strongly that immunization may be an important contributing factor in the permanent suppression of the lymphosarcoma transplants. In the riboflavin deficient rat antibody formation is unimpaired whereas immune responses in pyri-

1 Stoerk, H. C., *J. Biol. Chem.*, 1947, **171**, 437.

2 Emerson, G. A., and Tishler, M., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 184.

3 Emerson, G. A., Wurtz, E., and Johnson, O. H., *J. Biol. Chem.*, 1945, **160**, 165.

* The tumor was obtained through the courtesy of Dr. W. U. Gardner, Yale University, New Haven, Conn.

TABLE I.
Growth of Lymphosarcoma Implants and of Survival in the Various Experimental Groups.

Group	No. of mice	No. of days on defic. diet	μg fed		Tumor size in cm³ and survival in % at									
			B_2	Anti-B_2	10 days		20 days		30 days		40 days		50 days		60 days	
					cm³	%	cm³	%	cm³	%	cm³	%	cm³	%	cm³	%
I	51	—	60*	0	1.4	100	9.2	86	—	0		0		0		0
II	8	14	0	0	1.5	100	<1	100	0	37	0	37	0	37	0	37
III	10	26	0	0	0.8	100	0	100	0	90	0	70	0	60	0	30
IV	10	38	5	0	1.0	100	4.9	100	7.1	70	0	40	0	20	0	20
V	10	28	10	0	0.9	100	12.6	100	14.8	70	—	0	.	0	—	0
VI	10	14	3	500‡	2.5	100	<1	100	0	60	0	50	0	40	0	40
VII	10	14	100	500	1.3	100	11.5	90	—	0	—	0	-	0		0
VIII	10	20	8	1000§	—†	100	14.0	90	<1	70	0	30	0	30	0	30
IX	10	20	8	0	—†	100	15.3	90	15.8	10	—	0		0	—	0

* Complete natural diet.
† No measurement made.
‡ Isoriboflavin.
§ Galactoflavin.

doxine deficiency are suppressed.[4] It is probable that the recurrence of the tumor transplants following recovery from vitamin B_6 deficiency is due to the absence of immune bodies directed against the neoplastic tissue or its causal agent.

While pyridoxine deficiency produces marked atrophy of both normal[5] and neoplastic lymphoid tissue,[1] riboflavin deficiency affects only neoplastic lymphoid tissue. Riboflavin deficiency in the rat,[5] chick and mouse,[6] does not alter normal lymphoid tissue to a greater extent than does a comparable degree of inanition. The apparent exceedingly high requirement of neoplastic lymphoid tissue for vitamin B_2 may therefore be either a characteristic of the tumor lymphocyte or may perhaps represent a property of its causal agent, possibly a virus. The demonstration by Morris[7] that the growth of a mammary adenocarcinoma, related to a filtrable factor, is retarded by riboflavin deficiency, appears compatible with the latter possibility.

Summary. Marked regression of established lymphosarcoma (6 C3H-ED) implants occurred in all of 48 C3H mice rendered temporarily deficient in riboflavin either by the feeding of a diet low in this vitamin or by the administration of an antagonist. In most cases survival was significantly prolonged and 15 mice survived without recurrence of the tumors for more than 200 days. When animals which had survived 60 days or more were reinoculated with lymphosarcoma tissue, the second implant failed to take. Established lymphosarcoma implants in 81 control mice on a diet supplemented with adequate amounts of riboflavin, grew continuously and killed all animals within about 4 weeks.

[4] Stoerk, H. C., Eisen, H. N., and John, H. M., *J. Exp. Med.*, 1947, **85**, 365.

[5] Stoerk, H. C., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 90.

[6] Unpublished observations.

[7] Morris, H. P., *Ann. N. Y. Acad. of Sciences*, 1947, **49**, 119.