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REGRESSION OF PLISS LYMPHOSARCOMA IN RATS WITH HYPOVITAMINOSIS A AND SOME BIOCHEMICAL PARAMETERS

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Many investigations showing that vitamin A and its derivatives have anticarcinogenic activity [10] and that retinoids inhibit growth of some transplantable tumors [6, 13, 14] have been published in recent years. Meanwhile the effect of vitamin A deficiency on growth of experimental tumors has not been adequately studied. Early investigations showed [8] that in rats kept on a diet deficient in vitamin A the rate of growth of several sarcomas and carcinomas is reduced, but the vitamin status of the animal was not evaluated in these experiments. The results of our own experiments in which, under strictly controlled conditions, it was shown that Pliss lymphosarcoma (PLS) in rats in a state of hypovitaminosis A undergoes complete regression, and that retinoic acid (RA), unlike retinol, does not support tumor growth, are described below.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 150-160 g were used. The animals were given food and water ad lib. The main experiments were carried out on 60 rats divided into three equal groups. The rats were kept on an artificial diet, including 20% casein, heated to 120°C for 48 h to destroy vitamin A [2], 67% potato starch, 5% refined sunflower oil, 3% of mixed salt [5], 4.6% of autoclaved bakers' yeast, 0.1% inositol, 0.2% choline chloride, and essential vitamins [11] except vitamin A. Rats of group 1 (control) additionally received 500 μg of retinyl acetate with 0.2 ml of sunflower oil once a week perorally. Rats of group 2 did not receive retinyl acetate. Rats of group 3 received 200 μg of the methyl ester of RA on alternate days with 0.2 ml sunflower oil. After 2 months half the rats of each group were inoculated subcutaneously with PLS by the standard method [1], using material obtained after a single passage of the tumor in rats in a state of hypovitaminosis A. On the 8th day after inoculation of the tumor these rats were killed for biochemical tests. During the first 7 days after inoculation of PLS, instead of the methyl ester of RA the rats of group 3 received 100 μg of [11-^{14}C]-RA (specific radioactivity 160 μCi/mmole) daily, perorally, with 0.2 ml of sunflower oil.

Amino acids extracted from blood plasma with 70% methanol were determined on the Liquimat III amino-acid analyzer (Labotron, West Germany), under standard conditions recommended by the firm (the authors are grateful for technical help to G. S. Kaloshina, Junior Scientific Assistant at the A. I. Bakh Institute of Biochemistry, Academy of Sciences of the USSR).

The concentrations of retinyl palmitate (RP) and retinol in liver and PLS tissue (calculated per gram wet weight) were determined spectrophotometrically after preliminary fractionation of the extract by thin-layer chromatography on aluminum hydroxide [3], and the identity of the corresponding fractions and their quantitative proportions were confirmed by radioactive labeling (preliminary injection of [11-^{14}C]retinol into the animals). Labeled RA and its derivatives, extracted from the tissues with a mixture of chloroform and methanol (1:1), were

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TABLE 1. Growth of PLS in Rats Depending on Vitamin A Intake

Group of rats and experimental conditions	Rat No.	Size of tumor, cm^3		Content of vitamin A and its derivatives		
				in liver		in blood serum
		6th day	8th day	RP, μg/g	retinol, μg/g	retinol, ng/ml
1) Diet supplemented by vitamin A (control)	1	9,5	19,8	23,41	3,66	342
	2	9,5	24,3	25,58	2,21	291
	3	5,5	13,5	15,88	1,62	353
	4	8,7	18,4	23,08	1,79	385
	5	1,8	0,0	15,00	1,71	314
	6	9,5	23,1	53,76	2,26	537
	7	6,1	15,0	26,48	2,71	412
	8	15,2	24,3	52,63	2,32	526
	9	23,2	25,9	48,91	2,17	489
2) Diet without vitamin A	1	0,5	0,0	0,11	0,14	138
	2	0,1	0,0	0,11	0,11	114
	3	0,4	0,0	0,16	0,14	130
	4	1,5	0,0	0,27	0,20	93
	5	2,4	0,0	0,22	0,28	90
	6	0,2	0,0	0,21	0,14	69
	7	5,3	0,0	0,31	0,23	77
	8	5,4	0,0	0,19	0,25	88
	9*	8,1	17,6	0,22	1,11	278
	10*	14,5	23,5	0,17	1,01	281
3) Diet supplemented by RA and its ester	1	0,2	0,0	0,78	0,26	66
	2	0,1	0,0	0,31	0,25	63
	3	1,3	0,0	0,26	0,19	90
	4	1,9	0,0	0,70	0,26	116
	5	2,2	0,0	0,47	0,27	93
	6	1,8	0,0	0,45	0,18	95
	7*	5,6	5,3	1,76	0,29	165
	8*	6,7	9,4	2,00	0,39	167
	9*	9,2	10,4	1,87	0,30	153
	10*	9,5	11,9	3,76	0,59	161

Legend. Dimensions of tumors and vitamin A content given on 8th day after transplantation of lymphosarcoma. In rats Nos. 7-10 of group 3, on 8th day after transplantation of tumor RA content in liver and PLS tissue averaged 0.04 and 0.03 μg/g respectively, whereas content of esterified form of RA was 0.01 and 0.08 μg/g respectively. Asterisk indicates much milder hypovitaminosis A than in other rats of the given group.

determined after chromatographic fractionation on silica-gel in a solvent system of hexane—diethyl ether—acetic acid (7:3:1). Fractions containing label were eluted with a mixture of diethyl ether and acetic acid (5:1) and concentrations of RA and its derivatives (calculated as RA) were determined with an Intertechnique SL-4000 (France) liquid scintillation counter in standard ZhS-8 dioxane scintillator. In the calculations, specific radioactivity of RA was taken to be the same as initially, for under normal conditions RA is present in the tissues in negligible quantities. The serum retinol concentration was determined by high-pressure liquid chromatography on a Spectra Physics SP-8000 instrument with RP-8 column in a solvent system of acetonitrile—water, using retinol C_{19}-aldehyde, added to the test sample of blood serum in a quantity of 1 μg, as the internal standard (for technical help with these analyses the authors are grateful to M. V. Kaganovich, engineer at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). Retinyl acetate used in the work was of Soviet origin,

the methyl ester of RA was prepared from retinal by the method in [4], the [11-^{14}C]retinol (specific radioactivity 16 mCi/mmole) and [11-^{14}C]-RA (specific radioactivity 16 mCi/mmole) were from Amersham Corporation (England), neutral aluminum hydroxide (Brockman-II) was from Reanal (Hungary), with the addition of 10% water, and the L-5/40 μ silica-gel was from Chemapol (Czechoslovakia).

EXPERIMENTAL RESULTS

Preliminary experiments on rats receiving the ordinary animal house diet showed that RA, given perorally to the animals daily in a dose of 1 mg, had no appreciable effect on growth of PLS, although it accumulated not only in the liver, but also in the tumor.

Data showing growth of PLS in rats compared with differences in vitamin status are given in Table 1. It can be concluded from analysis of the results that regression of the lymphosarcoma in rats correlates distinctly with a low retinol concentration in the serum (below 140 ng/ml) and in the liver (below 0.36 μg/g tissue), but did not correlate with the RP content in the liver.

The fact will be noted that in most rats receiving an artificial diet supplemented with RA and its methyl ester, PLS regressed (Table 1, group 3, rats Nos. 1-6). It can be concluded from these facts that RA, which penetrated into the PLS cells sufficiently well, did not maintain growth of these cells, unlike retinol. The results must be compared with the fact that RA, while maintaining growth of animals with avitaminosis A, does not prevent atrophy of the testes in retinol deficiency, and only partially replaces retinol during regeneration of the liver in rats after partial hepatectomy [8].

Slow growth of tumors in some rats of group 3 (Nos. 7-10) can evidently be explained by the relatively high retinol concentration in the blood serum and liver of these animals. The action of RA, conserving vitamin A, was demonstrated previously in biological experiments [9, 12].

Incidentally, by the time of transplantation of the tumor (60th day of the experiment) growth of rats in groups 2 and 3 was virtually at the control level, and fertility in these groups (mean number of offspring per female after mating of one male with five females for 7 days) was only 20% lower than in the control. Regression of PLS in most rats of groups 2 and 3 thus indicates that malignant cells of this type exhibit high sensitivity to retinol deficiency in the animals. This can partly be attributed to the fact that, as our observations show, PLS cells cannot store retinol in the form of RP, and they contain retinol in concentrations 10-20 times lower than liver tissue (in rats of group 1 the retinol concentration in the lymphosarcoma was 0.1-0.2 μg/g tissue).

An important cause of regression of PLS in rats with vitamin A deficiency could be the low free amino acid level discovered in their blood plasma. On the 65th and 80th days of the experiment in rats of group 2 there was a decrease in both relative and absolute concentrations of arginine on average by 70-80% and a decrease in the absolute concentrations of methionine and leucine on average by 50-70%. In rats of group 3, which received RA instead of retinol, there was a sharp fall in the total amino acid content and a considerable fall (on average by 50%) in the arginine fraction. This sharp fall in the concentrations of essential amino acids in the blood plasma could be an important factor limiting the viability of actively proliferating malignant cells.

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