UTILIZATION OF PYRIMIDINE PRECURSORS IN TUMORS AND LIVER OF MICE WITH EHRLICH'S ASCITES CARCINOMA

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UDC 616.36-006.6-008.938.5-092.9-073.916

Investigation of the incorporation of labeled pyrimidine precursors into free pyrimidine nucleotides, RNA, and DNA of the liver and tumor cells from mice with Ehrlich's ascites carcinoma showed that the incorporation of C^{14}-uridine and C^{14}-orotic acid into the liver of tumor-carrying animals is reduced compared with that into the liver of healthy animals.

A factor inhibiting DNA synthesis in tumor cells has been found in the normal liver [5, 6]. In turn, considerable changes in the biosynthesis of nucleotides and nucleic acids have been found in the liver of animals with tumors [1, 3, 4, 7].

The incorporation of C^{14}-bicarbonate, C^{14}-orotic acid, C^{14}-uridine, C^{14}-thymidine, C^{14}-cytosine, and C^{14}-thymine into free pyrimidine nucleotides, RNA and DNA of the liver and tumor cells of mice with Ehrlich's ascites carcinoma and also of the liver of healthy mice was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 20-22 g. The labeled compounds were injected in doses of 1 μCi/g body weight. Fractions of free nucleotide monophosphates, RNA, and DNA were isolated from the liver and tumors by the method of Bresnick et al. [2], and their radioactivity was measured with a liquid scintillation counter (Nuclear Chicago) using a dioxane scintillator. The results relating to incorporation of labeled precursors are the mean values for three animals.

EXPERIMENTAL RESULTS

Incorporation of 2-C^{14}-orotic acid into the free pyrimidine nucleotides of the liver of mice with various tumors (Table 1) was 2-3 times greater than into the pyrimidine nucleotides of the tumors. Approximately the same difference was found when the radioactivities of RNA and DNA were measured.

After intravenous (Table 2) and intraperitoneal (Table 1) injection of 2-C^{14}-orotic acid the difference between incorporation of the label into pyrimidine nucleotides, RNA, and DNA of the liver of mice with Ehrlich's ascites carcinoma and into ascites cells remained and increased 36 and 72 h after injection. Incorporation of 2-C^{14}-orotic acid into these fractions of the liver of healthy mice (Table 2) was 2-3 times greater than into the livers of mice with Ehrlich's ascites carcinoma.

The utilization of different radioactive pyrimidine precursors in the liver and ascites cells of Ehrlich's carcinoma is compared in Table 3. Incorporation of C^{14}-uridine into the pyrimidine nucleotides, RNA, and DNA of the liver of tumor bearing mice was several times less than into the pyrimidine nucleotides, RNA, and DNA of the ascites cells. Incorporation of 2-C^{14}-uridine into free pyrimidine nucleotides of the healthy liver was greater than into the pyrimidine nucleotides of the ascites cells, whereas incor-

Department of Biochemistry, Central Postgraduate Medical Institute, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 12, pp. 66-69, December, 1970. Original article submitted February 11, 1970.

TABLE 1. Incorporation of 2-C^{14}-Orotic Acid into Free Pyrimidine Nucleotides, RNA, and DNA of Liver and Tumor of Tumor-Bearing Mice after Intraperitoneal Injection (M ± m)

Type of tumor	Exposure of label (in h)	Tumor investigated	Pyimidine nucleotides	RNA	DNA
			pulses/min/mg tissue		
Subcutaneous melanoma	1	Liver Tumor	1041,3±84,1 552,7±51,1 $P<0,05$	120,1±13,8 36,5±3,1 $P<0,05$	32,0±4,7 7,8±0,9 $P<0,05$
Ehrlich's intramuscular carcinoma	1	Liver Tumor	1061,0±140,0 293,1±35,4 $P<0,05$	99,2±10,4 28,8±3,1 $P<0,05$	13,0±1,7 8,0±0,4 $P>0,05$
Crocker's tumor (C-180)	1	Liver Tumor	380,4±43,5 105,1±12,0 $P<0,05$	21,0±3,3 1,6±0,2 $P<0,05$	2,1±0,2 1,0±0,1 $P<0,05$
Ehrlich's intraperitoneal carcinoma	1	Liver Tumor	1152,8±118,1 305,6±36,7 $P<0,05$	105,7±17,4 22,0±2,3 $P<0,05$	15,5±1,5 6,5±0,6 $P<0,05$
	36	Liver Tumor	102,5±15,2 13,2±1,4 $P<0,05$	548,5±66,2 60,1±9,0 $P<0,02$	21,9±2,5 2,3±0,3 $P<0,02$
	72	Liver Tumor	77,8±10,5 12,7±1,0 $P<0,05$	306,0±49,8 49,4±6,9 $P<0,05$	33,8±5,2 15,7±2,0 $P>0,05$

TABLE 2. Incorporation of 2-C^{14}-Orotic Acid into Free Pyrimidine Nucleotides, RNA, and DNA of Liver and Ehrlich's Ascites Carcinoma of Mice after Intravenous Injection (M ± m)

Exposure of label (in h)	Animals	Tissue investigated	Pyrimidine nucleotides	RNA	DNA
			pulses/min/mg tissue		
1	Healthy Tumor-bearing	Liver Liver Tumor	1794,2±124,0 952,9±49,5 233,8±15,7 $P_1<0,01$ $P_2<0,001$	367,6±23,6 126,5±12,5 13,4±0,9 $P_1<0,001$ $P_2<0,001$	164,1±17,1 110,6±6,5 18,3±1,5 $P_1<0,05$ $P_2<0,001$
36	Healthy Tumor-bearing	Liver Liver Tumor	133,5±9,0 43,6±3,3 7,5±0,6 $P_1<0,001$ $P_2<0,001$	991,9±48,9 852,4±63,2 51,1±3,9 $P_1>0,1$ $P_2<0,001$	250,9±16,4 165,9±11,7 23,6±1,5 $P_1<0,01$ $P_2<0,001$
72	Healthy Tumor-bearing	Liver Liver Tumor	40,4±3,6 16,5±1,3 2,1±0,2 $P_1<0,001$ $P_2<0,001$	476,4±30,8 484,0±26,6 18,6±1,8 P_1 — $P_2<0,001$	175,1±15,5 98,2±6,6 11,1±1,4 $P_1<0,002$ $P_2<0,001$

poration into nucleic acids, on the other hand, was several times less. Incorporation of tritiated 5-CH_3-thymidine into all three fractions was maximal in ascites cells and minimal in the liver of healthy mice.

Following injection of 2-C^{14}-cytosine the radioactivity of all three fractions of ascites cells was also maximal compared with the radioactivity of these fractions in the liver. Incorporation of 2-C^{14}-thymine showed no significant difference on comparison of all three fractions.

It can be concluded from the results given in Tables 1 and 2 that the biosynthesis of pyrimidine nucleotides de novo in Ehrlich's ascites carcinoma cells is 2-3 times less than in the liver of tumor-bearing mice, and in the latter it is 2-3 times less than in the liver of healthy mice (Table 2). Since the incorporation of 2-C^{14}-orotic acid into RNA and DNA of ascites cells was reduced by about the same degree as into

TABLE 3. Incorporation of Pyrimidine Precursors into Pyrimidine Nucleotides, RNA, and DNA of Liver and Ehrlich's Ascites Carcinoma of Mice 36 h after Intraperitoneal Injection (M ± m)

Labeled precursors	Animals	Tissue investigated	Pyrimidine nucleotides		RNA		DNA	
			pulses/min/mg tissue	pulses/min/A 260*	pulses/min/mg tissue	pulses/min/μg RNA	pulses/min/mg tissue	pulses/min/μg DNA
$NaHC^{14}O_3$	Healthy	Liver	8,4±0,4	381±24	20,3±1,6	3,0±0,2	5,9±0,3	1,7±0,13
	Tumor-bearing	Liver	4,5±0,4	239±26	15,9±1,4	1,4±0,16	7,4±0,8	1,9±0,20
		Tumor	0,2±0,02	12±2	17,8±1,2	1,7±0,18	6,8±0,5	1,4±0,17
			$P_1<0,001$	$P_1<0,001$	$P_1>0,05$	$P_1<0,001$	$P_1>0,1$	$P_1>0,1$
			$P_2<0,001$	$P_2<0,002$	$P_2>0,1$	$P_2>0,1$	$P_2>0,1$	$P_2>0,1$
C^{14}-uridine	Healthy	Liver	217,7±18,8	7 250±646	689,8±43,0	71,6±4,8	87,6±4,1	24,3±1,7
	Tumor-bearing	Liver	17,5±0,8	895±86	98,8±6,6	17,9±1,2	26,9±1,6	9,5±0,52
		Tumor	134,3±9,6	6 395±429	796,3±82,8	174,1±14,6	752,6±35,6	98,8±9,4
			$P_1<0,001$	$P_1<0,001$	$P_1<0,001$	$P_1<0,001$	$P_1<0,001$	$P_1<0,001$
			$P_2<0,001$	$P_2<0,001$	$P_2<0,001$	$P_2<0,001$	$P_2<0,001$	$P_2<0,001$
Tritiated 5-methylthymidine	Healthy	Liver	568,0±42,6	26 115±2212	13,4±0,9	2,2±0,16	92,2±4,3	30,7±2,5
	Tumor-bearing	Liver	716,7±77,5	30 759±2274	20,2±1,7	2,5±0,16	274,7±14,7	70,4±4,1
		Tumor	906,5±56,5	48 426±3417	31,3±2,0	3,5±0,25	1116,4±56,6	151,7±7,4
			$P_1>0,1$	$P_1>0,1$	$P_1<0,05$	$P_1>0,1$	$P_1<0,001$	$P_1<0,002$
			$P_2>0,1$	$P_2<0,02$	$P_2<0,02$	$P_2<0,05$	$P_2<0,001$	$P_2<0,001$
C^{14}-cytosine	Healthy	Liver	5,7±0,5	202±23	2,5±0,1	0,5±0,03	1,6±0,12	0,4±0,04
	Tumor-bearing	Liver	1,6±0,06	37±4	1,9±0,1	0,3±0,02	3,8±0,3	1,0±0,14
		Tumor	10,0±0,5	245±31	11,7±0,5	1,6±0,11	31,0±3,3	4,3±0,31
			$P_1<0,001$	$P_1<0,001$	$P_1<0,05$	$P_1<0,02$	$P_1<0,002$	$P_1<0,002$
			$P_2<0,001$	$P_2<0,001$	$P_2<0,001$	$P_2<0,001$	$P_2<0,002$	$P_2<0,001$
C^{14}-thymine	Healthy	Liver	6,6±0,6	233±37	16,3±1,6	2,1±0,22	9,3±0,83	2,2±0,14
	Tumor-bearing	Liver	6,6±0,3	233±27	7,1±0 4	1,1±0,15	6,3±0,44	2,0±0,12
		Tumor	5,1±0,5	186±34	6,6±0,4	1,6±0,10	26,9±1,3	5,6±0,24
			P_1	P_1	$P_1<0,01$	$P_1<0,02$	$P_1<0,05$	$P_1>0,1$
			$P_2>0,05$	$P_2>0,1$	$P_2>0,1$	$P_2>0,05$	$P_2<0,001$	$P_2<0,001$

*Optical density of fraction at 260 mμ.

pyrimidine nucleotides, there are no grounds for the conclusion that biosynthesis of nucleic acids in ascites cells is at a lower level than in the liver. This is confirmed by experiments with $NaHC^{14}O_3$ (Table 3), which demonstrated no significant difference between the incorporation of $C^{14}O_2$ into nucleic acids of the liver and ascites cells.

The results of experiments with 2-C^{14}-uridine and tritiated 5-methylthymidine and with 2-C^{14}-cytosine and 2-C^{14}-thymine indicate that ascites cells are better able to utilize preformed precursors for nucleic acid synthesis.

The fact that incorporation of 2-C^{14}-uridine, 2-C^{14}-orotic acid, and $NaHC^{14}O_3$ into free pyrimidine nucleotides of the liver of tumor-bearing mice is many times smaller than their incorporation into the liver of healthy mice is evidently attributable to a decrease in the intensity of biosynthesis of conjugated forms of pyrimidine nucleotides (UDP-glucose, CDP-choline, and so on) in the liver of tumor-bearing animals because of inhibition of the specific functions of the liver.

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