CHANGES IN RELATIONS BETWEEN TWO PATHWAYS OF SYNTHESIS OF RNA PRECURSORS IN THE TISSUES OF ANIMALS WITH FAST GROWING HEPATOMAS

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The ratio between the de novo and "reserve" pathways of synthesis of pyrimidine nucleotides of RNA changes in the de novo direction in the spleen, thymus, and liver of mice and rats with fast growing transplantable hepatomas. This phenomenon is due to a sharp decrease in uridine utilization by the tissues of the tumor-bearing animal, for the tumor utilizes mainly this precursor to synthesize RNA. This observation is regarded as the result of successful competition between the tumor and the host tissues for vitally important metabolites, one form of systemic action of the malignant tumor.

KEY WORDS: RNA synthesis; "reserve" pathway; fast-growing hepatoma; systemic action of tumors; immunocompetent organs.

The common pyrimidine precursor of RNA synthesis – uridine monophosphate (UMP) – is known to be synthesized in animal cells in two different ways: from simple precursors such as CO_2, NH_3, and aspartic acid, with the formation of orotic acid as intermediate metabolite (the de novo pathway) or by addition of ribosyl pyrophosphate to uracil with the formation of uridine and its subsequent phosphorylation to the nucleotide (the "reserve" pathway). The relationship between these two pathways of UMP synthesis in animal and human tissues varies, but the "reserve" pathway of UMP synthesis in fast-growing malignant tumors becomes predominant [6].

In the investigation described below the relationship between the de novo and "reserve" pathways was studied in normal tissues of mice and rats, in hepatomas, and in the tissues of animals with the tumor.

EXPERIMENTAL METHOD

Experiments were carried out on C_3HA mice with transplantable hepatoma 22A [1] and Wistar rats with Zajdela hepatoma. Both hepatomas are of the ascites kind and are detectable 5-7 days after transplantation. Uridine-^{14}C and orotic acid-^{14}C were injected intraperitoneally 60 min before the animals were killed in equimolar quantities into each group of three animals: mice in a dose of 1.3 μmole (specific activity $5.5 \cdot 10^7$ cpm/μmole), rats in a dose of 1.72 μmole (specific activity $5.0 \cdot 10^8$ cpm/μmole). The animals were killed by decapitation. The liver was perfused with cold physiological saline. The tissues were minced and pressed through a metal sieve. Ascites hepatoma cells were sedimented by centrifugation at 2000g and washed twice or three times to remove erythrocytes at 700g for 3 min, after which the solid cell residue was obtained by centrifugation under the original conditions. Tissue samples weighing 100-200 mg were extracted with 0.5 N $HClO_4$ for 20 min at 4°C. The residue was then washed three times successively in 5% and 2.5% TCA and in water. To remove phospholipids the residues were treated with ethanol, an ethanol–ether (3 : 1) mixture at 60°C for 15 min, and with ether at 35°C for 10 min. RNA and DNA were separated by the method of Schmidt and Thannhauser [4]. RNA was determined quantitatively by Spirin's spectrophotometric method [2]. Radioactivity was counted in ZhS-8 scintillator on a Mark 2 counter (Nuclear Chicago, USA).

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TABLE 1. Incorporation of ^{14}C-Precursors into RNA of Various Tissues of Normal C_3HA Mice and Mice with Hepatoma 22A (M ± m)

Tissue	Specific activity of RNA, counts/min/mg RNA		Orotic acid/uridine
	orotic acid	uridine	
Liver:			
Normal	16 183±840	14 913±2 556	1,1
Animal with tumor	14 368±2520	1 941±230	7,4
Spleen:			
Normal	12 358±1290	57 167±2 880	0,22
Animal with tumor	13 902±4600	2 080±356	6,7
Thymus:			
Normal	1 976±195	9 030±626	0,21
Animal with tumor	3 260±224	2 114±340	1,5
Hepatoma 22A:			
5th day	8 164±509	153 172±7 600	0,05
8th day	5 177±612	100 325±25 195	

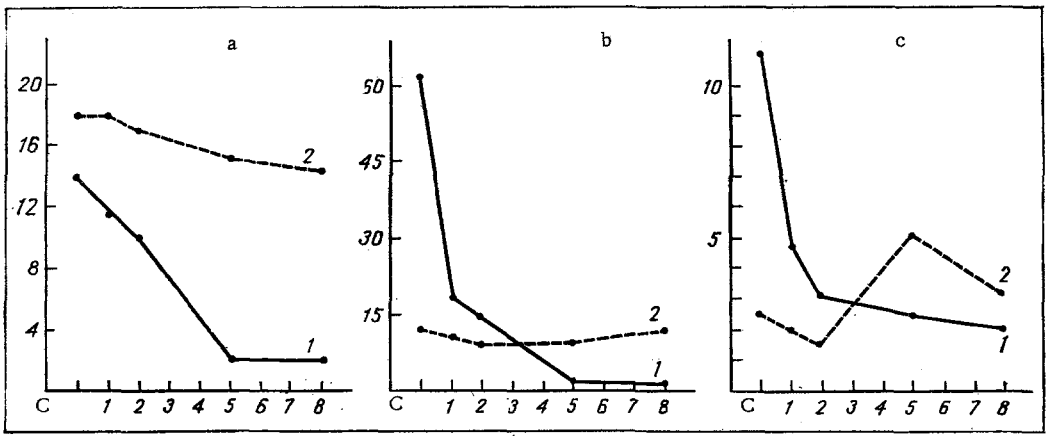

Fig. 1. Incorporation of uridine-^{14}C (curve 1) and orotic acid-^{14}C (curve 2) into RNA of liver (a), spleen (b), and thymus (c) of normal mice and mice with hepatoma 22A. Abscissa, growth of hepatoma (in days), ordinate, specific activity of RNA [in counts/min/mg RNA ($\times 10^3$)]. C) Control.

EXPERIMENTAL RESULTS

Data showing the incorporation of orotic acid and uridine into RNA of the liver, spleen, and thymus of normal mice and mice with hepatoma 22A on the 8th day of its growth are given in Table 1.

In the normal liver the ratio between incorporation of orotic acid and uridine was 1.1. The spleen and thymus are organs in which predominantly uridine is used as RNA precursor. However, the utilization of this precursor by the hepatoma (5th and 8th days) was considerably in excess of the normal tissues and the orotic acid/uridine ratio was 0.05, i.e., the tumor utilized uridine four times more actively than the tissues of the spleen and thymus, in which this ratio was 0.21-0.22. A general tendency was observed in the tissues of mice with an ascites hepatoma for the orotic acid/uridine ratio to increase on account of a sharp decrease in the incorporation of uridine into RNA.

In the next experiments these changes were studied starting from the first day after transplantation of the hepatoma. As Fig. 1 shows, a sharp decrease in incorporation of uridine-^{14}C into RNA was observed in the liver and spleen although a relatively constant and high level of incorporation of orotic acid-^{14}C continued to be found. The decrease reached its lowest level on the 5th day. In the thymus, besides a marked decrease in incorporation of uridine into RNA, some increase in the incorporation of orotic acid also was found (Fig. 1). Hence it can be concluded that the tumor utilized uridine selectively for RNA synthesis and competed successfully for this substrate with the tissues of the host animal.

Data on the incorporation of orotic acid and uridine into RNA of the normal and regenerating liver and of the liver of rats with Zajdela hepatoma are given in Table 2.

TABLE 2. Relations Between Two Pathways of RNA Biosynthesis in Rat Tissues (M ± m)

Tissue	Incorporation into RNA, counts/min/mg RNA		Orotic acid/ uridine
	orotic acid-^{14}C	uridine-^{14}C	
Liver:			
Normal	138 493±15 230	6 375±385	22,0
Animal with tumor	96 569±22 507	2 666±620	32,0
Regenerating liver, 20 h	598 978±98 089	13 483±2 633	44,4
Zajdela hepatoma, 5th day	17 647±11 513	167 691±54 468	0,1

By contrast with the mouse liver, in normal rat liver orotic acid is the principal precursor for RNA synthesis: The orotic acid/uridine labeling ratio in RNA was 22.0. In the regenerating rat liver incorporation of orotic acid into RNA was increased almost fivefold, whereas incorporation of uridine was only doubled, i.e., the increase in RNA synthesis was mainly by the de novo pathway. In Zajdela hepatoma, just as in the mouse hepatoma, the orotic acid/uridine ratio was less than in all the tissues studied in these animals, namely, 0.1. A decrease in the incorporation of uridine into RNA by 60% compared with the normal level was found in the liver of an animal with Zajdela hepatoma, but incorporation of orotic acid also was significantly reduced, although not to the same degree, i.e., by 30%. This last observation is in agreement with data in the literature [5]. The ratio of incorporation of orotic acid/uridine into RNA was 32.0, higher than in normal liver. Despite species differences in the relationship between the metabolic pathways for synthesis of RNA precursors, it is thus clear that the growth of highly malignant hepatomas in mice and rats prevents normal RNA synthesis, especially in immunocompetent organs.

The interception of nucleic acid precursors by the tumor in the lymphoid tissues of mice and rats also extends, as the writers have shown [3], to thymidine, with the result that DNA synthesis also is sharply inhibited in these organs. The results described above indicate the biochemical processes which may lie at the basis of the immunodepressive action characteristic of malignant tumors.

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