COMPARATIVE RATE OF UTILIZATION OF OROTIC ACID AND URIDINE BY HUMAN AND RAT BONE MARROW CELLS DURING SHORT-TERM CULTIVATION

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Experiments on human and rat bone marrow cells in culture showed that the efficiency of synthesis of pyrimidine nucleotides by the emergency route (from C^{14}-uridine) is several times higher than the efficiency of its synthesis de novo (from C^{14}-orotic acid).

Experiments on cultures of human and rat bone marrow cells have shown that the efficiency of synthesis of pyrimidine nucleotides by the emergency route (from C^{14}-uridine) is several times higher than the efficiency of their synthesis de novo (from C^{14}-orotic acid). The bone marrow has very limited ability to synthesize purines [5, 6, 8, 9]. Biosynthesis of pyrimidines de novo evidently does take place in the bone marrow, but it is not the principal way for the activity of enzymes such as dihydro-orotic acid dehydrogenase is very low. It is also known that about half of the requirement of thymidyl nucleotides in the bone marrow is provided by reutilization of thymidine [3].

The relative importance of the pathways of pyrimidine biosynthesis from C^{14}-orotic acid (de novo) and from C^{14}-uridine (emergency route) in bone marrow cells were studied in this investigation. These results may be of interest both for the development of pathogenetic treatment of hematopoietic disturbances and also for bone marrow conservation.

EXPERIMENTAL METHOD

Human bone marrow was prepared in solution No. 3 of the Central Institute of Blood Transfusion in the ratio 1:1. Any erythrocytes present were removed by decanting the suspension of bone marrow cells from the residue of erythrocytes formed after 30 min. The procedure was repeated twice. The bone marrow cells were sedimented by centrifuging and suspended in incubation medium [45% Eagle's medium, 45% of a 0.5% lactalbumin hydrolyzate in Hanks' solution, 10% group AB (IV) serum, and 50 mg Na_2EDTA to 100 ml medium]. Each incubation sample contained $4 \cdot 10^6$ cells in a volume of 2 ml. Incorporation of uniformly labeled C^{14}-Asp, 2-C^{14}-Oro, and 2-C^{14}-Urd* into RNA and DNA of the bone marrow cells was investigated. The quantity of isotope added to the sample was: C^{14}-Asp, 2 μCi, and 2-C^{14}-Oro and 2-C^{14}-Urd, 5 μCi each. Incubation continued for 18 h.

TABLE 1. Utilization of C^{14}-Asp, 2-C^{14}-Oro, and 2-C^{14}-Urd during 18 h for Nucleic Acid Synthesis in a Culture of Human Bone Marrow (ppm/μg)

Expt. No.	Precursor	RNA	DNA
1	C^{14}-Asp	25,8±3,6	9,3±0,7
	2-C^{14}-Oro	136,3±9,0	41,9±6,1
	2-C^{14}-Urd	333,7±19,0	83,6±13,2
P		<0,001	<0,02
2	2-C^{14}-Oro	200±11,0	38,7±2,6
	2-C^{14}-Urd	472±45	71,8±7,7
P		<0,001	<0,01

In the experiments on bone marrow cells, male Wistar rats weighing 200-250 g were decapitated. The bone marrow

*Asp – aspartic acid, Oro – orotic acid, Urd – uridine.

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TABLE 2. Incorporation of 2-C^{14}-Urd and 2-C^{14}-Oro into Free Pyrimidine Nucleotides, RNA, and DNA in Culture of Rat Bone Marrow Cells during Incubation for 1.5 h (in pulses/min from a preparation obtained from the total number of cells)

No. of cells in sample (mills)	Precursor	Free pyrimidine nucleotides			RNA			DNA		
		I	II	III	I	II	III	I	II	III
4	2-C^{14}-Urd	18 787	26 228	28 169	2593±44	3072±94	4355±79	308±11	460±18	638±28
	2-C^{14}-Oro	1 359	5 663	19 246	27±8	351±14	1719±27	0	81±15	363±14
8	2-C^{14}. Urd	31 934	29 422	60 123	4509±139	6733±411	7019+203	845±34	1067±51	1341±111
	2-C^{14}. Oro	1 478	6 079	28 697	37±8	333±17	1324±102	0	64±14	337±51
16	2-C^{14}. Urd	53 533	—	—	7549±341	—	—	1620±106	—	—
	2-C^{14}. Oro	2 825	6 963	37 409	146±10	595±95	2885±398	18±6	133±10	995±230
50	2-C^{14}. Urd	89 192	—	—	26493±1752	—	—	—	—	—
	2-C^{14}. Oro	5 821	19 978	70 356	486±66	1681±119	7036±635	—	—	—

Legend: I, II, III) 0.5, 2, and 8 μCi per sample, respectively.

was flushed out of the femora with incubation medium [90% medium No. 199, 10% group AB (IV) serum, and 0.3 mg heparin to each 100 ml medium]. The incubation samples contained 4, 8, 16, and 50 million cells in 2 ml. 2-C^{14}-Urd and 2-C^{14}-Oro were added to samples containing this number of cells in doses of 0.5, 2, and 8 μCi. The cells were incubated at 1.5 h at 37°C, and then washed three times with cold 0.9% NaCl solution. Incorporation into three pyrimidine nucleotides, RNA, and DNA was investigated. The mean values of the results of three parallel experiments were analyzed. The specific activity of the preparations was: 2-C^{14}-Urd, 8 μCi/μmole; 2-C^{14}-Oro, 25 μCi/μmole; and C^{14} Asp, 80 μCi/μmole.

Isolation of the Three Pyrimidine Nucleotides, RNA, and DNA. The three pyrimidine nucleotides were isolated by the method of Bresnick [2]. RNA was hydrolyzed in 2 ml 0.3 N KOH at 37°C for 18 h. DNA and protein were precipitated with 0.25 ml 5 N $HClO_4$ and the residue was washed twice with 0.5 ml 0.5 N $HClO_4$. DNA was hydrolyzed in 2 ml 0.5 N $HClO_4$ in an ultrathermostat at 90°C for 15 min. The residue was washed with 0.5-1.0 ml 0.5 N $HClO_4$. The content of RNA and DNA was determined spectrophotometrically. Radioactivity of the samples was measured on a Nuclear Chicago Mark 1 liquid scintillation counter.

EXPERIMENTAL

As was pointed out above, the sample of 2-C^{14}-Oro had a specific activity three times higher than 2-C^{14}-Urd, and this must have led to greater utilization of 2-C^{14}-Oro than of 2-C^{14}-Urd by the bone marrow cells if the de novo and emergency routes were equally efficient. However, as the results in Table 1 show, incorporation of 2-C^{14}-Oro into RNA was only one-third, and into DNA only one-half of incorporation of 2-C^{14}-Urd during incubation of a culture of human bone marrow cells for 18 h.

To rule out any effect of differences in the supply of endogenous Oro and Urd on their utilization, experiments were carried out with different numbers of rat bone marrow cells and different quantities of labeled precursor (Table 2). The results showed, first, that 2-C^{14}-Urd is utilized many times more than 2-C^{14}-Oro. Second, they showed that the incorporation of 2-C^{14}-Urd increased almost in proportion to the number of cells and, consequently, uridine was present in excess in the medium and no significant dilution with endogenous unlabeled Urd took place. Incorporation of 2-C^{14}-Oro increased by a much smaller degree than the number of cells. Third, the results showed that an increase of 4 and 16 times in the content of 2-C^{14}-Urd was accompanied by only a slight increase in the incorporation of 2-C^{14}-Urd into free pyrimidines, RNA, and DNA, whereas an increase in the content of 2-C^{14}-Oro led to a virtually linear increase in the activity of free pyrimidines, RNA, and DNA.

The results of these experiments can be considered from the standpoint of the ratio between the enzyme and substrate concentrations. By increasing the number of cells in the sample, the concentration of enzymes catalyzing the conversion of Oro and Urd was increased. By changing the quantity of labeled Oro and Urd added, the substrate concentration was changed. Consequently, the concentration of Urd created by a dose of 0.5 μCi was close to the saturating concentration for all samples with the different numbers of cells. The velocity of the reaction catalyzed by uridine kinase was near to its maximum with this concentration of Urd. In the case of Oro this saturation concentration could not be found. The velocity of the reaction catalyzed by orotidine-5-phosphate pyrophosphorylase did not reach its maximum even with the highest concentration (8 μCi) of 2-C^{14}-Oro. The results of these experiments to compare the incorporation of C^{14}-Urd and C^{14}-Oro with equal specific activity of the preparations (8 μCi/μmole) also revealed a lower level of 2-C^{14}-Oro incorporation than when its specific activity was 25 μCi/μmole. This indicates that the increase in the velocity of the Oro utilization reaction produced by an increase in substrate concentration is abolished by threefold dilution of the label. The results in Tables 1 and 2 indicate that the de novo pathway in human and rat bone marrow is much less efficient than the utilization of pyrimidine nucleosides, whether liberated by breakdown of the nucleic acids of the corresponding tissue or transported from other organs possessing a greater capacity for de novo synthesis (among which the liver plays an important role), for the synthesis of pyrimidine nucleotides.

The two forms of uridine kinase found by Krystal [4] are evidence of differences in the ability of tissues to utilize nucleosides. Uridine kinase I, with a molecular weight of 120,000, is dominant in the adult liver and in slowly growing tumors. Uridine kinase II, with a molecular weight of 30,000 , is predominant in rapidly proliferating tissues. Matsushita et al. [7] investigated the relationship between the synthesis of pyrimidine nucleotides from H^3-Oro and H^3-Urd in the heart muscle of rats and found that this tissue was better able to utilize H^3-Urd. The writers have shown that Ehrlich's ascites tumor of mice is more able to incorporate C^{14}-uridine and H^3-thymidine than the liver of healthy mice and the liver of mice with tumors, but is less able to utilize C^{14}-Oro.

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