IDENTIFICATION AND QUANTITATIVE STUDY OF URIDINEDIPHOSPHATE-ACETYLGLUCOSAMINE IN HEALTHY AND LEUKEMIC HUMAN LEUKOCYTES

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The content of uridinediphosphate-acetylglucosamine (UDPAG) in leukocytes of patients with chronic myeloid leukemia and polycythemia is 2.1 and 2.8 times greater respectively than in healthy persons. In chronic lymphatic leukemia the UDPAG content in the leukocytes in normal.

UDPAG* occupies a central position in the interconversions of aminosugars and is a precursor of the hexosamine components of mucoid compounds [6, 14, 15] whose presence has been demonstrated in human leukocytes [4, 5, 7, 13]. Meanwhile, very little is yet known regarding the quantitative content of UDPAG in the leukocytes [12].

In connection with the important function of UDPAG in the synthesis of mucopolysaccharides and the established fact that the quantitative content of mucoid compounds differs in healthy human leukocytes and leukocytes of leukemia patients [3, 10, 11], in the present investigation we studied the quantitative content of UDPAG in these cells.

EXPERIMENTAL METHOD

The test object consisted of leukocytes from the circulating blood of healthy persons and of patients with leukemias and polycythemia. The morphological characteristics of leukocytes, the methods of precipitating them from the blood and of isolating nucleotides from these cells have been described previously [1, 2, 9]. The location of UDPAG stains on the chromatogram was determined in an Ultrachemiscope, and compared with the R_f value of a standard preparation (Sigama, USA). Stains located at the witness level were cut out and eluted with water for 30-60 min. UDPAG in the eluate was determined quantitatively from the amount of UDP formed after acid hydrolysis. The eluate was hydrolyzed in 0.03N H_2SO_4 for 10 min at 100° and neutralized to pH 7.4. UDP in the hydrolysate was determined by the pyruvate kinase – lactate dehydrogenase reaction from the decrease in absorption of light at 340 mμ [8]. The process took place in two stages in accordance with the scheme:

1. PEP + UDP $\xrightarrow{\text{pyruvate kinase}}$ pyruvate + UDP
2. Pyruvate + NADP-H_2 $\xrightarrow{\text{lactate dehydrogenase}}$ lactate + NADP.

The experimental mixture (3 ml) contained 2.5 ml hydrolysate, 150 μmoles Tris-buffer (pH 7.4), 20 μmoles $MgCl_2$, 20 μmoles NaF, 2.2 μmoles PEP, 0.6 μmole NADP-H_2, and 0.25 mg crystalline pyruvate kinase (Reanal), with which lactate dehydrogenase contained in the pyruvate kinase as an impurity, was introduced. NADP-H_2 was added only to the experimental samples. The reaction was carried out at room temperature (20°). The amount of UDP formed as a result of hydrolysis of UDPAG was calculated from the formula:

$$\frac{V \cdot \Delta E}{6.22} = \text{UDP } \mu\text{moles},$$

where V is the volume of the cuvette and ΔE the change in extinction.

*The following abbreviations are used in this paper: UDPAG is uridinediphosphate-acetylglucosamine, UDPG is uridinediphosphate-glucose, UDP is uridinediphosphate, PEP is phosphenolpyruvate, NADP-H_2 is reduced phosphonicotinamide-adenine nucleotide.

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TABLE 1. Quantitative Content of UDPAG (in μmoles/ml leukocytes) in Leukocytes of Healthy Persons and of Patients with Leukemias and Polycythemia

Healthy subjects	Chronic myeloid leukemia	Chronic lymphatic leukemia	Polycythemia
28,0±2,7	60,4±4,2	29,3±3,7	79,6±4,1
—	$P<0,001$	$P>0,5$	$P<0,001$

EXPERIMENTAL RESULTS

As Table 1 shows, the highest content of uridine nucleotide in the leukocytes was found in chronic myeloid leukemia and polycythemia: 2.1 and 2.8 times higher than normal respectively. No difference was found in the UDPAG content in leukocytes of healthy persons and of patients with chronic lymphatic leukemia.

The absence of relationship between UDPAG content in the leukocytes and their mucopolysaccharide content will be apparent. In the lymphocytes of patients with chronic lymphatic leukemia, for example, the content of acid mucopolysaccharides was much smaller than normally [3], while the UDPAG concentration was equal to that of healthy human leukocytes; in chronic myeloid leukemia the acid mucopolysaccharide level was much higher than in polycythemia, while the UDPAG content was lower.

It is interesting to note that in our previous investigation we found the same absence of relationship between the UDPG content in the leukocytes and the content of glycogen, a polysaccharide synthesized with the participation of UDPG. It may be considered either that the biosynthesis of glycogen and mucopolysaccharides is not the main channel for the formation and utilization of UDPAG and UDPG in the leukocytes, or that the rate of metabolism of these coenzymes varies in cells with different polysaccharide contents.

LITERATURE CITED

1. A. D. Vladimirova and I. F. Seits, Byul. Éksperirm Biol. i Med., No. 10, 56 (1964).
2. I. F. Seits, I. S. Luganova, and A. D. Vladimirova, Biokhimiya, No. 2, 295 (1963).
3. M. F. Kharchenko, A. I. Blinova, and I. F. Seits, in: Problems in Hematology [in Russian], Leningrad (1966), p. 65.
4. A. Ferrara and G. Cousoli, Progr. Med. (Napoli), 8, 209 (1952).
5. D. Gigante and S. I. Magalini, Haematologica, 47, 293 (1962).
6. L. Glaser and D. Brown, Proc. Nat. Acad. Sci. (Wash.), 41, 5 (1955).
7. G. P. Kerby, J. Clin. Invest., 34, 1738 (1955).
8. A. Kornberg, Methods in Enzymology, 1, 435 (1955).
9. L. F. Leloir, J. Biol. Chem., 203, 1005 (1955).
10. S. I. Magalini and G. Mascioli, J. Lab. Clin. Med., 162, 961 (1963).
11. S. Moshides, M. Stefanini, S. I. Magalini, et al., J. Clin. Invest., 37, 127 (1958).
12. R. N. Ondarsa, Acta Physiol. Lat.-Amer., 10, 129 (1960).
13. E. Orlandi, N. Benda, and G. Mengini, Progr. Med. (Napoli), 11, 101 (1955).
14. T. N. Pattabiraman and B. K. Bachdamat, Biochim. Biophys. Acta, 51, 129 (1961).
15. S. Suzuki, J. Biol. Chem., 237, 1393 (1962).