BIOCHEMISTRY AND BIOPHYSICS

THE NUCLEIC ACIDS, PHOSPHOLIPIDS AND PHOSPHOPROTEINS OF THE HUMAN LEUCOCYTE

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The biochemical study of leucocytes is continuously expanding to include almost all aspects of the metabolism of these cells, which are of utmost importance in the maintenance of the normal state of the animal body. Nevertheless, the data presently available on the white blood cells are both incomplete and contradictory. This is particularly true for the acid-insoluble phosphorus compounds of the leucocytes, namely, the nucleic acids, phospholipids and phosphoproteins. Certain data, even though contradictory, dealing with the quantitative content of nucleic acids and phosphoproteins in the leucocytes, have recently been published [3, 4, 6] but there is practically no information on the turnover rates of these compounds.

It was shown in a previous communication from this laboratory [1] that a powerful glycolytic mechanism for the splitting of sugar existed in the human leucocyte, and that this mechanism could completely satisfy the cell's energetic requirement under anaerobic conditions. The extent of glycolysis in the granulocytes is so large, that it is not completely suppressed in the presence of atmospheric oxygen, as is the case with other cells, and subsequently, under normal physiological conditions, respiration and glycolysis coexist. Furthermore, respiration was shown to be partially suppressed in the presence of intensified glycolysis, with the result that oxygen uptake was lower in the presence of glucose than in its absence (so-called reverse Pasteur effect) [1]. These peculiarities in the metabolism of leucocytes set them metabolically apart from other cells of the animal body, and are reminiscent of the metabolic patterns of malignant cells.

However, the observations listed above dealt but with the very basis of the energy metabolism – respiration, glycolysis and adenosinetriphosphate (ATP) turnover. It was considered of importance to gain further insight into the biochemical characteristics of the human white blood cells, and primarily of the facets of metabolism concerned with their growth and function. Since the cellular synthetic activity, growth, division, as well as some other functions, are connected with the turnover of nucleic acids and, possibly, of other acid-insoluble organophosphates, we have undertaken a study of the quantitative content and the turnover rates of the phosphorus moiety of the ribonucleic acids (RNA), desoxyribonucleic acids (DNA), phospholipids (PL) and phosphoproteins (PP) in the various types of human leucocytes, in normal subjects and in certain pathological conditions. Corresponding investigations were carried out with highly purified leucocyte suspensions from normal human subjects, and from patients suffering from both chronic and acute myeloid leukemia, and from chronic lymphadenosis.

EXPERIMENTAL METHODS

Leucocyte suspensions in human serum containing no more than 10% red blood cells were incubated with shaking in the presence of glucose and radioactive phosphate (360,000 cpm per ml of incubate) in Warburg vessels at 37°, under aerobic and anaerobic conditions. At the end of the incubation period the cells were separated and washed, and analyzed for inorganic phosphate, ATP, RNA, DNA, PL and PP. The nucleotides

The Quantitative Content of the Phosphorus of Various Compounds in the Human Leucocyte and Their Turnover Rates

	Phosphorus													
	ATP		RNA			DNA	DNA		PL			PP		
				RSA			RSA			RSA			RSA	
	γ	RSA	γ	I	II	γ	I	II	γ	I	II	γ	I	II
1. Normal donor														
Aerobic	636±22	63±5	1191±51	3,3±0,7	5,9±0,2	4235±278	0,17±0,03	0,25±0,05	2 60±135	2,4±0,4	2,7±0,5	96±14	10±2,6	13∓3,1
Anaerobic	636±26	62±5	1165±134	4,8±0,5	6,4±1,0	3960±283	0,20±0,03	0,31±0,04	2490±235	1,7±0,3	1,9±0,3	102±20	12±1,7	18±3,0
1. Chronic myeloid leukemia														
Aerobic	621±36	71±3,5	1950±179	3,0±0,6	3,9±0,05	4351±299	0,17±0,02	0,23±0,02	2570±230	0,8±0,3	1,3±0,2	111±30	10,1±1,9	14,9±2,5
Anaerobic	644±38	70±4,6	1780±193	3,8±0,6	4,7±0,6	4900±336	0,14±0,01	0,17±0,02	2360±189	1,2±0,2	1,2±0,3	106±24	11,5±1,3	15,6±1,2
2. Acute myeloid														
Aerobic	740±66	65±5,6	3590±453	1,8±0,2	2,6±0,4	6750±440	0,13±0,02	0,15±0,02	—	—	—	167±45	14±2,6	18±2,4
Anaerobic	760±72	64±5,2	3150±420	2,1±0,4	2,6±0,4	6580±600	0,11±0,02	0,14±0,02	—	—	—	187±25	11±1,5	14±1,6
3. Lymphadenosis														
Aerobic	736±40	83 5,4	4043±210	2,3±0,3	3,0±0,11	12940±595	0,25±0,08	0,31±0,06	2943±318	1,0±0,06	2,1±0,8	143±24	13,5±1,7	18±2,4
Anaerobic	780±47	83 9,2	3530±100	2,4±0,2	3,0±0,4	12980±920	0,34±0,1	0,39±0,1	2875±319	1,4±0,07	1,5±0,6	131±17	15,8±1,9	22,4±3,7

Values expressed as γ phosphorus per 1 g of dry cell weight. Incubation time, 60 min. temperature 37°; cell suspensions in serum.

$$\text{RSA I} = \frac{\text{Specific activity of the fraction phosphorus}}{\text{Specific activity of the inorganic phosphorus}}; \quad \text{RSA II} = \frac{\text{Specific activity of the fraction phosphorus}}{\text{Specific activity of the ATP-phosphorus}}$$

[2] Incubation time — 30 min.

(ATP) were adsorbed onto charcoal [2], the acid-insoluble compounds were fractionated according to Schmidt-Thannhauser [5]. The specific activity (SA) of the phosphorus of all the fractions mentioned was determined, and the relative specific activity (RSA) of the fractions was calculated as follows: I – with respect to intracellular inorganic phosphorus (RSA-I); II – with respect to the phosphorus of the immediate precursor of most of the compounds investigated – ATP (RSA-II).

EXPERIMENTAL RESULTS

Presented in the Table are the results of the quantitative assays and turnover rate determinations on the phosphorus of ATP, RNA, DNA, PL and PP in various groups of human leucocytes, incubated for one hour at 37°, in human serum under aerobic and anaerobic conditions (as an exception from general procedure, the leucocytes from patients suffering from acute myeloid leukemia were incubated for 30 min in view of their elevated metabolic activity).

It is noteworthy, with respect to the content of the various organophosphates in the leucocytes, that there was little deviation in the PP and PL content of leucocytes in the various groups investigated. On the other hand, the RNA phosphorus content varied widely, its lymphocyte content being twice that of leucocytes of chronic myeloid leukemia patients, and nearly four times that of normal human leucocytes. The DNA-phosphorus content of normal leucocytes was approximately the same as that of leuocyctes of chronic myeloid leukemia patients. The DNA-phosphorus content of lymphocytes was found to be very high, being about three times that of the normal leucocytes. This is quite understandable in view of the mass ratio of the nucleus and the whole cell in lymphocytes. There was a considerable increase in the RNA-phosphorus content, and some increase in the DNA-phosphorus content (compared with the normal leucocytes) in the acute leukemia group. No significant differences between the ATP contents of the various cell groups were found.

It is obvious from the results of the tracer experiments that the least metabolically active fraction was that of DNA; the RSA value of its phosphorus was less than one per cent of the intracellular inorganic phosphorus, and only slightly higher when calculated on the basis of ATP-phosphorus. The rate of phosphorus rejuvenation in the PL fraction was found to be considerably higher, the RSA value being 1-2.4%. The RNA phosphorus was found to exchange at still higher rates, the RSA values obtained in the various leucocyte groups, ranging from 1.8 to 4.8%. The rate of the PP-phosphorus exchange was found to be exceptionally high. The RSA values of this fraction reached 10-15%.

Of all the leucocyte groups investigated, the normal leucocytes showed the highest degree of phosphorus turnover in the RNA and PL fractions, while the cells obtained from lymphadenosis patients showed the highest turnover of phosphorus in the DNA and PP fractions. The rate of ATP-phosphorus turnover was highest in the leucocytes taken from acute leukemia patients, and slightly lower in the lymphocytes.

It was mentioned above that the metabolism of all the human leucocyte types under anaerobic conditions was energetically equivalent to their metabolism in the presence of air. This has now found confirmation in the present study of the behavior of the complex intracellular phosphorus-containing compounds of the leucocyte – nucleic acids, PL and PP. As will be seen from the Table, the rate of phosphorus turnover in these compounds was found to be practically the same under aerobic and anaerobic conditions. The glycolytic cleavage of sugar in the absence of air was apparently capable to support energetically the complete resynthesis of even the most complex high molecular weight compounds of the human white blood cells.

The effective ATP resynthesis, and the satisfactory turnover of the nucleic acid-, PL- and PP-phosphorus in the leucocytes under anaerobic conditions demonstrated the excellent adaptability of these cells to possible oxygen deficiency, and their ability to carry out completely the necessary specific functions under conditions of deficiency, or complete absence, of atmospheric oxygen.

SUMMARY

Leucocytes in a healthy man contain the following (measured in gamma of phosphorus per 1 g dry weight of the cells): ribonucleic acid – 1191 ± 51; desoxyribonucleic acid – 4235 ± 278; phospholipids – 2760 ± ± 135; phosphoproteins – 96 ± 14. Leucocytes from the blood of patients with chronic myeloid leukemia contain correspondingly with the above: 1950 ± 179; 435 ± 299; 257 ± 230 and 111 ± 30. The quantity of phosphorus in the same compounds of leucocytes in the patients with chronic lymphadenosis equals 4043 ± 210;

12,940 ± 595; 2943 ± 318 and 143 ± 24, respectively. In acute leukemia: RNA – 3590 ± 453; DNA – 6750 ± 440; phosphoproteins – 167 ± 45. Phosphorus exchange is most intense in phosphoproteins and the least so in DNA, while RNA and phospholipids occupy a middle position. The rapidity of phosphorus exchange in all of these compounds does not depend on the conditions of aeration. Resynthesis of adenosinetriphosphate and phosphorus exchange in nucleic acids, phospholipids, and of phosphoproteins in leucocytes is carried out in anaerobic conditions in the same way as in aerobic.

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*See English translation.