INVESTIGATION OF ERYTHROCYTE RNA IN LEUKEMIA

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The RNA and total protein content in erythrocytes of patients with leukemia and healthy donors were investigated by paper chromatography, electrophoresis, spectrophotometry, and other methods. The RNA content in erythrocytes of leukemic patients is increased while the total protein content is within normal limits. The base ratio of the RNA fluctuates considerably in leukemia.

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The results of our previous investigations showed that erythrocytes of patients with leukemia have an increased content of nucleic acid and of free adenylic and guanylic acids [2].

In the present investigation the RNA content was studied in the erythrocytes of patients with leukemia.

EXPERIMENTAL METHOD

The RNA content was determined spectrophotometrically in a mixture of ribonucleotides of alkaline hydrolysates. Hydrolysates were obtained from RNA isolated preparatively from washed erythrocytes of leukemic patients and healthy donors by the method of Schmidt and Thannhauser with slight modifications. For this purpose the erythrocytes were homogenized in perchloric acid, with homogenate was centrifuged, and the residue containing RNA were washed and treated by alkaline hydrolysis in 0.5 N KOH for 18 h at 37°. The content of each of the 4 mononucleotides composing RNA was also determined in the alkaline hydrolysates. The total protein content of the erythrocytes was also determined.

To determine the separate nucleotides composing the RNA, the alkaline hydrolysates obtained from the preparations were freed from ClO_4 ions and fractionation of the nucleotides was carried out by electrophoresis and chromatography on paper. Improved electrophoretic fractionation was obtained by the use of Markham and Smith's method [4, 5]. These workers recommend preliminary treatment of the paper strip with 2% acetic acid solution. During passage of the electric current the paper is cooled by immersion in an intermediate vessel containing carbon tetrachloride between the two vessels containing the electrodes. Electrophoresis continues for 5-6 h at 900 V. For fractionation 0.05-0.1 ml of hydrolysate was applied to the paper strip. Stains of cytidylic, adenylic, g guanylic, and uridylic acids were developed in the Ultrachemiscope after electrophoresis and drying. The stains were cut out, cut into small pieces, and eluted in 5 ml citrate-phosphate buffer (citric acid and Na_2HPO_4; pH 3.0, 18 h, 37°). The content of nucleotides in the eluates was determined spectrophotometrically at the following wavelengths for each nucleotide respectively: 270, 260, and 255 mμ (maximum) and 290 mμ (minimum of absorption for all nucleotides). The nucleotide content in the stain was calculated from the coefficients given in [1]. To obtain a more precise successive distribution of the nucleotide stains in electrophoresis, standard mixtures of these compounds were separated electrophoretically.

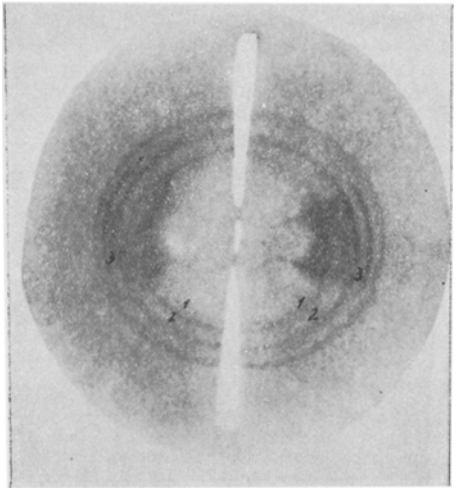

Fig. 1. RNA of erythrocytes from patients (right) and donors (left). 1) adenylic acid; 2) guanylic acid; 3) pyrimidine nucleotides.

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TABLE 1. RNA and Protein Content (μg/g Erythrocytes)

Donors		Patients	
RNA	protein	RNA	protein
417	250	953	212
420	275	603	255
330	225	660	170
562	220	1 000	300
564	250	1 270	205
501	290	912	360
463	250	670	212
426	275	660	300
400	290	806	295
562	290	751	365
424	210	660	250
$M \pm m$			
460,8±25	256,8±9	813±69	265±19

By means of radial chromatography (Gabermann's method [3]) four bands were obtained corresponding to the individual nucleotides composing RNA. Paper for chromatography was preliminary treated with 0.5% EDTA solution alkalified to pH 8.5 and washed with distilled water. The stains were developed by a mixture of a saturated solution of benzidine and 0.05 M KI solution (2:1) and quickly photographed.

EXPERIMENTAL RESULTS

An increase in the RNA content was found in the erythrocytes of patients with chronic leukemias. The total protein content was determined by the biuret reaction. A solution of casein in 0.1% sodium acetate was used for the standard curve. The protein content varied in erythrocytes of both donors and patients, but no significant differences were obtained between them (Table 1).

The base ratio of RNA (A + C/G + U) for healthy donors in these experiments was relatively constant, ranging from 0.8 to 1, while in the patients it varied considerably, from 0.5 to 1.5.

Chromatographic fractionation of nucleotides obtained from the RNA preparations revealed the presence of purine and pyrimidine nucleotides. The order of the stains from the point of application of the samples was: adenylic acid, guanylic acid, pyrimidine nucleotides (Fig. 1).

Qualitative changes in the enzyme protein may perhaps be observed in the erythrocytes of patients with leukemia. This hypothesis is confirmed by studies of the ratio between activity of lactate dehydrogenase isoenzymes in the erythrocytes of leukemic patients [6]. The number of leukocytes contaminating the samples of erythrocytes from the patients was checked. The figure was very low and was no higher than that in the donors.

LITERATURE CITED

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