THE URIDINE DIPHOSPHOGLUCOSE CONTENT OF LEUCOCYTES IN HEALTHY AND IN LEUKEMIC INDIVIDUALS

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In recent years reports have appeared that glycogen synthesis in liver, muscle, brain and other tissues, is implemented through the participation of uridine diphosphoglucose (UDPG) [5, 8, 9]. Recently, data have been obtained on the importance of the uridine diphosphate mechanism for glycogen synthesis in human leucocytes and thrombocytes [1 3]. In this process UDPG fulfills the important function of glycoside-residue donor. It is known, however, that the coenzyme functions of UDPG are not limited to participation in glycogen synthesis. It has been established that this nucleotide is an important cofactor in enzymatic reactions connected with the reduction and synthesis of mono-, di-, and trisaccharides in animal and plant tissues; UDPG plays an important role as precursor of UDPG-glucuronic acid in protective synthesis in the formation of mucopolysaccharides [14, 15].

We know of only one study [12] in which, together with other free nucleotides, the UDPG content in the blood of healthy individuals and those with chronic myeloid leukemia was investigated. The study was made not on isolated leucocytes but on the entire cell mass, whereas the UDPG content in chronic myeloid leukemia was determined in only 2 cases. Data on the content of this nucleotide in leucocytes in chronic lymphadenosis and in polycythemia are lacking.

In connection with the important function of UDPG in glycogen synthesis and the earlier established fact of different glycogen concentrations in leucocytes of healthy individuals from patients with polycytheimia and leukemias [1],it is of interest to study the quantitative UDPG content in these cells. Our paper has elucidated a comparative study of the quantitative UDPG content and the rate of turnover of its glycolytic component in the leucocytes of healthy individuals, patients with chronic myelosis, chronic lymphadenosis and polycythemia.

METHODS

With polycythemia, in the peripheral blood of observed patients about 90% of the leucocytes consist of cells of the myeloid series, being predominantly segmented and metamyelocyte forms; in chronic myeloid leukemia the blood contains up to 93% of myeloid cells of different degrees of maturation, the quantity of myeloblasts being less than 3%; in chronic lymphadenosis not less than 95% of the white cells are lymphocytes. The leucocyte are isolated in a gelatin-citrate solution [4], maintained in physiological solution containing 0.1% glucose with subsequent centrifugation. The volume of the leucocyte precipitate was measured and the cells were used for preparing a suspension.

Two series of experiments were performed. In series I,the UDPG content was measured directly in isolated leucocytes; in series II,the content and rate of turnover of UDPG were studied after a preliminary 60 min incubation of the leukocytes with C^{14}-glucose at 37° in a mixture of albumin and Ringer's phosphate according to Krebs (1:1).

The UDPG content in leucocytes was determined in the following manner. The centrifuged leucocytes were suspended in physiological solution and the nucleotides were extracted with chloric acid (final concentration 2%) for 20 min in the cold. The supernatant liquid obtained after centrifugation was brought to pH 2 using a 2N NaOH

TABLE 1. UDPG Content in Leucocytes from Healthy Individuals and Patients with Polycythemia, Chronic Myeloid Leukemia and Chronic Lymphadenosis (in μg per ml of leucocytes)

No. of Expt.	Healthy individuals	Patients		
		Chronic myeloid leukemia	Polycythemia	Chronic lymphadenosis
1	22.0	40.0	22.0	13.0
2	23.8	35.0	22.0	16.0
3	20.0	34.0	20.0	17.0
4	22.0	35.0	19.0	25.0
5	20.0	40.0	21.0	17.0
6	21.0	38.0	20.9	–
M±m	21.5±0.7	37.0±1.0	20.8±0.6	17.6±1.7

TABLE 2. Content and Rate of Metabolism of UDPG in Leucocytes from Healthy Individuals and Patients with White Cell Dyscrasias and Polycythemia after Incubation with C^{14}-Glucose

Group	UDPG isolated per ml of cells	
	Quantity (in μg)	Radioactivity (counts per min)
Healthy	19.3	768
	20.2	806
	20.7	857
Patients with polycythemia	20.4	768
	19.3	778
	20.5	750
Patients with chronic myeloid leukemia	37.0	1655
	37.4	1555
	34.0	1469
Patients with chronic lymphadenosis	19.0	791
	17.5	744
	15.6	648

solution. The nucleotide extract was adsorbed with activated charcoal which had previously been treated with a 3% solution of hexyl alcohol to improve its adsorptive properties [11]. Adsorption took place for 30 min at 0°. The complete adsorption of nucleotides was verified by using the light absorption at 260 millimicrons. The charcoal bearing the adsorbed nucleotides was washed with cold 0.001 N HCl to remove salts which interfere with the chromatographic separation of the nucleotides. The nucleotides were eluted by washing down 3 times with 50% ethyl alcohol containing 0.02 M NH_4OH. The combined eluates were evaporated at 30° in a vacuum (5 mm) to a small volume and the concentrated solution was studied chromatographically. For separation of nucleotides a method of descending chromatography was used, with BF-11 paper and a solvent system of 1 M ammonium acetate (pH 7.5) and ethyl alcohol in ratio of 30:75 [13]. The usual chromatographic separation took 28 h, after which the chromatogram was dried and the localized spots of nucleotides were assayed in the ultrachemiscope. The spots which appeared at the level of the marker were cut out and eluted with 2 portions of distilled water (2 + 1 ml) for 30 min in the cold. Light absorption at 260 and 280 millimicrons was studied in the eluate thus obtained.

Quantitative assay for UDPG was performed on the same eluate using the UDPG-pyrophosphorylase reaction. The experimental mixture used for this quantitative assay contained 2.5 ml of eluate from the chromatogram spots in 3 ml, 150 micromoles of tris-buffer (pH 7.4), 5 micromoles of cysteine, 20 micromoles of $MgCl_2$, 0.5 mg of crystalline phosphoglucomutase, 0.05 ml of UDPG-pyrophosphorylase (0.2 mg protein), 1 micromole triphosphopyridinenucleotide (TPN) and 0.05 ml of glucose-6-phosphate dehydrogenase (0.1 mg protein). After obtaining several stable readings on the system we introduced 1 micromole of pyrophosphate. The reaction took place at room temperature (20°). Control samples differed from the experimental in that an eluate was used not from the chromatogram spots but from equal-sized portions of other parts of the chromatogram paper which were free of nucleotide. Pyrophosphorelysis began almost immediately after the addition of the pyrophosphate in accordance with the reaction: UDPG + pyrophosphate $\rightleftharpoons$ uridinetriphosphate + glucose-1-phosphate. The glucose-1-phosphate which forms is converted by phosphoglucomutase to glucose-6-phosphate. The latter is oxidized by glucose-6-phosphate dehydrogenase: glucose-6-phosphate + TPN $\rightarrow$ 6-phosphogluconic acid + reduced TPN. The increase in light absorption at 340 millimicrons indicates the course of the UDPG-pyrophosphorylase reaction and, subsequently, the presence of UDPG in the extracts. In the given system it is possible quantitatively and exactly to titrate the entire contents in a standard UDPG solution. A preparation 99-100% pure gave extinction values of ΔE_{340} = 1.33 per μmole of UDPG.

The UDPG content and rate of its metabolism in leucocytes after preliminary incubation of the cells in C^{14}-glucose were determined in the following manner. Leucocytes (0.1 ml) were incubated in Ringer's phosphate with blood serum in the ratio 1:1 in the presence of uniformly labelled C^{14}-glucose (3 mg with total radioactivity of 450,000 counts/min). Versene (final concentration 0.1%) was used as anticoagulant. Samples were incubated for 30 min at 37°. After incubation, the cells were separated by centrifugation, washed with cold physiological solution and treated

with cold chloric acid. Further procedures in the isolation of UDPG were carried out as described above. After the eluate was obtained from the chromatogram spots, one ml was taken for determination of the UDPG radioactivity, and the remaining 2 ml were used in the UDPG-pyrophosphorylase reaction.

The following enzyme and co-enzyme preparations were used: phosphoglucomutase and TPN—Hungarian preparations from the firm Reanal; UDPG-pyrophosphorylase, obtained in the laboratory according to LePage [10]; glucose-6-phosphate dehydrogenase, according to Kornberg [6].

RESULTS

As shown in Table 1, there appear to be no essential differences in UDPG content between leucocytes from normal individuals and those from patients with polycythemia. The UDPG content is slightly lower in the lymphocytes of patients with chronic lymphadenosis. As for the leucocytes of patients with chronic myeloid leukemia, the UDPG content is 2 times greater than in the lymphocytes and about 1.8 times greater than in donor leucocytes.

It must be noted, however, that the many manipulations carried out to isolate UDPG from cells cause a considerable loss of this coenzyme. Therefore, the data included in Table 1, evidently, are too low. Special control experiments showed that with the use of our extraction, adsorption, elution, evaporation, and chromatography procedures about 50% of a standard added UDPG preparation is lost. Calculating from this recovery data, the UDPG content of human leucocytes must be reckoned as much higher (about 2 times).

The data presented in Table 2 show that upon incubation of the cells with C^{14}-glucose the radioactive marker is included in the UDPG. The experiments indicate that radioactivity of UDPG in leucocytes from patients with chronic myeloid leukemia is significantly greater than such in leucocytes of healthy individuals and patients with chronic lymphadenosis or polycythemia. However, if it is considered that the absolute UDPG content in leucocytes of patients with chronic myeloid leukemia is greater than in other white blood cells studied, then the specific radioactivity of glucose in the composition of UDPG from these cells almost exactly coincides with such in the composition of UDPG from other species of leucocytes, this value being very close to the specific radioactivity taken in the C^{14}-glucose experiment. Thus, upon incubation of leucocytes with C^{14}-glucose for 30 min, complete turnover of the glycolyl residues of the UDPG takes place, which indicates the high metabolic activity of this compound. It may be suggested that the higher UDPG content in leucocytes from patients with chronic myeloid leukemia is connected to a certain degree with the increased rate of glycogen turnover in these cells [2].

SUMMARY

A study was made of the quantitative content of uridine diphosphoglucose (UDPG) and the rate of renewal of its glycoside component in the leucocytes of healthy individuals, and in patients with chronic myelosis, chronic lymphadenosis and polycythemia.

In healthy individuals and in patients with polycythemia, chronic myelosis and chronic lymphadenosis the UDPG content per 1 ml of leukocytes was found to amount to 44, 42, 72, and 34 micrograms respectively. In studying the rate of glucose component metabolism in UDPG it was revealed that within 30 min of incubation the specific radioactivity of the UDPG glycoside and that of the medium glucose became entirely equivalent.

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