PHOSPHATE ESTERS OF HUMAN ERYTHROCYTES. IV. SEDOHEPTULOSE-1,7-DIPHOSPHATE, OCTULOSE-1,8-DIPHOSPHATE, INOSINE TRIPHOSPHATE AND URIDINE DIPHOSPHATE

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Although sedoheptulose-1,7-diphosphate (S-1,7-P)[1] and octulose-1,8-diphosphate (O-1,8-P) have been shown to accumulate during the metabolism of inosine by human erythrocytes (1-3), no evidence has been presented thus far for the occurrence of these phosphate esters in intact human erythrocytes. ITP has recently been reported present in relatively high concentrations in erythrocytes of two siblings of five members of a family (4); this characteristic appears to be linked to a genetic trait. During analysis of the "ITP zone" obtained by HVPE of neutral trichloroacetic extracts of erythrocytes of these subjects, an additional phosphate ester was found having no ultraviolet absorbing properties. The compound was identified as S-1,7-P (4). Extension of this work to "non-ITP" erythrocytes, revealed the presence of S-1,7-P and small amounts of ITP in normal fresh human erythrocytes. The subsequent search for O-1,8-P in fresh erythrocyte preparations also disclosed its presence and that of UDP.

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[1] The following abbreviations are used: DHAP, dihydroxyacetone phosphate. E-4-P, erythrose-4-phosphate. HVPE, high voltage paper electrophoresis. ITP, inosine triphosphate. O-1,8-P, octulose-1,8-diphosphate. R-5-P, ribose-5-phosphate. S-1,7-P, sedoheptulose-1,7-diphosphate. UDP, uridine diphosphate.

MATERIALS AND METHODS.

S-1,7-^{32}P and O-1,8-^{32}P were prepared as described by Vanderheiden (3). IT^{32}P and UD^{32}P were obtained by isotope equilibrium exchange with ^{32}Pi(AT^{32}P) (5).

Preparation of extracts. The trichloroacetic acid extract of fresh heparinized blood (four 10 ml aliquots) is prepared as described by Vanderheiden and Boszormenyi-Nagy (5). In this case however, the saline red cell suspension (6 ml) obtained after the saline wash, is mixed and treated directly with 19 ml of 10.5% trichloroacetic acid. The extract of the four samples is pooled, neutralized and lyophilized. Finally the dried material is dissolved in 1.0 ml of cold distilled water. The separation and analysis of the phosphate esters is carried out as described by Vanderheiden (3).

In order to facilitate the localization of the phosphate esters in HVPE sheets, known amounts of ^{32}P-labeled reference markers, (S-1,7-P - ITP, and O-1,8-P - UTP)[2] of high specific activity, are applied with the sample on the HVPE sheet prior to electrophoresis. After separation and detection of the radioactive bands, eluates from strips of the "S-1,7-P - ITP" and "O-1,8-P - UDP" zones obtained from fresh erythrocytes from various subjects, were further purified by ion exchange and (or) paper chromatography, and analyzed by enzymatic and spectrophotometric assay. ITP and S-1,7-P were separated by ion exchange chromatography in a column of Dowex 1 Cl x 10 (100-200 mesh) 0.5 cm diameter and 7 cm height. Paper chromatography was carried out on acid-washed Whatman #1 paper using isobutyric acid - 1 N NH_4OH - 0.1 M sodium EDTA (50:30:0.8) (Solvent I), or absolute ethanol - 1 M ammonium acetate, pH 3.8 - 0.1 M sodium EDTA, pH 8.2 (75:29:1) (Solvent II). The method described by Horecker (6) was used

[2] S-1,7-^{32}P and O-1,8-^{32}P, obtained by HVPE (from blood incubated with inosine and ^{32}Pi) of erythrocyte phosphate esters, contain small amounts of ^{32}P-labeled ITP and UDP. ITP is found with S-1,7-P, while UDP is found with O-1,8-P. The concentration of these compounds in the reference marker solutions is determined after separation by HVPE and paper chromatography as illustrated in fig 1, or by difference of the total phosphate and the phosphate equivalent calculated from the enzymatic assay of S-1,7-P and O-1,8-P.

for the enzymatic determination of S-1,7-P and O-1,8-P. Isotope dilution was also used to determine the concentration of the phosphate esters in question in some of the blood preparations.

RESULTS AND DISCUSSION.

The first indication of the existence of more than one compound in the S-1,7-P and O-1,8-P zones from HVPE was obtained in comparative analyses of these compounds in blood samples incubated with inosine (See Table 5, ref. 3); the total phosphate concentration of the eluates from the S-1,7-P and O-1,8-P zones was slightly higher than the phosphate values calculated from the DHAP assay of the products of aldolase cleavage of the respective diphosphates. The studies reported here confirm the fact that a different phosphate ester is present in each of the two HVPE zones. When the eluates from the S-1,7-P zone was subjected to paper chromatography in Solvent II, in addition to S-1,7-P (R_{pi}=0.36), an ultraviolet absorbing spot was observed with an R_{pi} (0.08) similar to that of ITP (See Fig 1); ion exchange chromatography also revealed two major components, one eluting with 0.05 N HCl (S-1,7-P), and the other (ultraviolet absorbing) eluting with 0.2 N HCl (ITP). The spectral characteristics of the second compound resembled those of hypoxanthine nucleotides. Similarly when the eluate from the O-1,8-P zone was subjected to paper chromatography in Solvent I, in addition to O-1,8-P (R_f=0.13) an ultraviolet absorbing compound was observed with an R_f (0.22) corresponding to that of UDP and with absorption spectrum characteristic of uracyl nucleotides.

The results of analysis of the different compounds obtained from HVPE of preparations of several samples of human erythrocytes, are shown in Table 1. The S-1,7-P concentrations determined in four different sets of conditions (See Table 1), either by enzymatic assay or by determination of phosphate, range from 0.4 to 3.9 µg P/g Hb with an average value of 1.7 µg P/g Hb. The values for ITP, obtained either by subtracting the S-1,7-P (determined enzymatically) from the total phosphate, or by determination of .

Table 1

Analysis of S-1,7-P and O-1,8-P HVPE zones.

Method of Separation	S-1,7-P				O-1,8-P			
	No. of Samples	Total P	S-1,7-P	ITP	No. of Samples	Total P	O-1,8-P	UDP
	μg P/g Hb				μg P/g Hb			
HVPE - Eluate	3	3.7-6.4			5	1.8-4.4		
HVPE - Eluate Enzymatic assay	7	2.8-6.3	0.4-3.9	0. -4.5[a]	4	2.8-3.9	0.5-1.1	1.9-1.8[a]
Ion exchange followed by paper chromatography	2		1.4-1.5		2		0.6-0.9	
Ion exchange	1[b]	2.9	1.9	1.0				
Paper chromatography (Isotope dilution)	2		0.4-0.5	0.7-0.8	2		0.2	0.6-0.9

a - Total phosphate minus phosphate equivalent from enzymatic assay.
b - Pooled sample from four subjects.

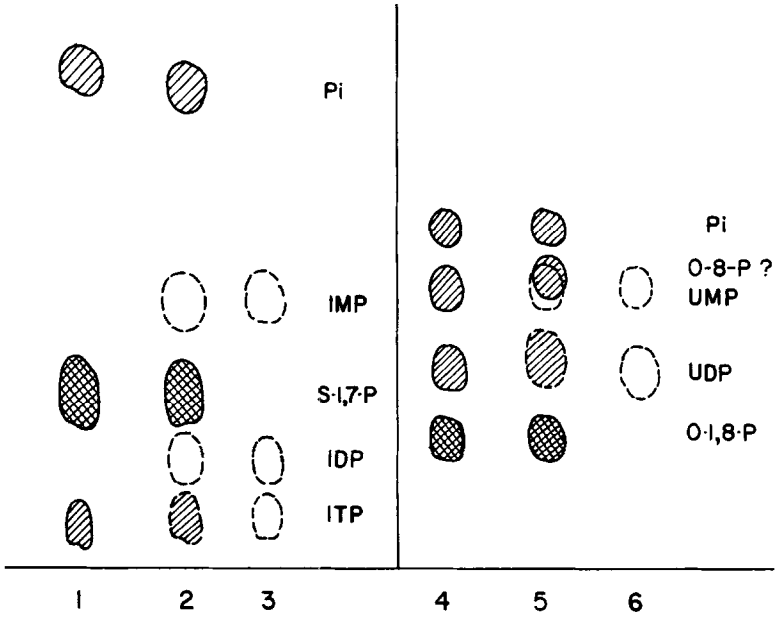

Figure 1

Separation of S-1,7-P from ITP, and O-1,8-P from UDP by paper chromatography. 1 - Eluate from the S-1,7-P zone from HVPE. 2 - Standard ITP plus 1. 3 - Standard ITP. (1-3) Solvent II. 4 - Eluate from the O-1,8-P zone from HVPE. 5 - Standard UDP plus 4. 6 - Standard UDP. (4-6) Solvent I. Radioactivity is indicated by cross-hatch areas; ultraviolet absorbing spots are indicated by broken lines. (Ascending chromatography on acid-washed Whatman #1, 16-18 hrs.).

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phosphate after separation of the two compounds, range from 0. to 4.5 μg P/g Hb, with an average value of 1.8 μg P/g Hb. In the case of O-1,8-P the values range from 0.2 to 1.1 μg P/g Hb, with an average value of 0.6 μg P/g Hb. The concentration of UDP ranges from 0.6 to 2.8 μg P/g Hb with an average value of 1.8 μg P/g Hb. These compounds (S-1,7-P, O-1,8-P, ITP and UDP) although present in relatively small concentrations, appear to be normal constituents of human erythrocytes.

From the present knowledge of erythrocyte metabolism it is difficult to assign a definite role to the sedoheptulose and octulose diphosphates. It may well be that they exist as trace side products of the aldolase reaction between DHAP and E-4-P and R-5-P (1), with no significance

in the overall metabolism of the erythrocyte. In the case of the nucleotides, UDP is of course an intermediate in the formation of cofactors essential for the isomerization of sugars. The role of ITP however, is still subject to speculation; it may represent vestigial traces of a cofactor essential in the metabolism of cells in the early stages of development.

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SUMMARY. Fresh human erythrocytes were found to contain sedoheptulose-1,7-diphosphate, octulose-1,8-diphosphate, inosine triphosphate and uridine diphosphate in average concentrations of 27,10,19, and 29 mμMoles/g Hb respectively.

REFERENCES.

1. Bucolo, G. and Bartlett, G.R., Biochem. Biophys. Res. Comms., 3, 620 (1960).
2. Bartlett, G.R. and Bucolo, G., Biochem. Biophys. Res. Comms., 3, 474 (1960).
3. Vanderheiden, B.S., Anal. Biochem., 8, 1 (1964).
4. Vanderheiden, B.S., Proc. Xth Congress Internal. Soc. Blood Transfusion, Stockholm, 1964; 544 (1964).
5. Vanderheiden, B.S. and Boszormenyi-Nagy, I., Anal. Biochem. (in press).
6. Horecker, B.L., in H.U. Bergmeyer (Editor), Methods of Enzymatic Analysis, Academic Press, Inc., New York, 1963, p. 111.