may also be recalled that ferrous iron (at the same optimal concentration of $M/800$) considerably reversed the "dilution effect" found with intact cells[4]. These findings suggest that perhaps a further electron-transporting system, in addition to the menadione and cytochrome systems, is a component of the nitrate reductase system of *E. coli* 1433.

S. D. WAINWRIGHT

Department of Microbiology, Yale University, New Haven, Conn. (U.S.A.)

[1] M. R. POLLOCK, *Brit. J. Exptl. Pathol.*, 27 (1946) 419.
[2] D. J. D. NICHOLAS, A. NASON AND W. D. MCELROY, *Nature*, 172 (1953) 34.
[3] D. J. D. NICHOLAS AND A. NASON, *J. Bacteriol.*, 69 (1955) 580.
[4] S. D. WAINWRIGHT AND M. R. POLLOCK, *Brit. J. Exptl. Pathol.*, 30 (1949) 190.
[5] W. D. WOSILAIT AND A. NASON, *J. Biol. Chem.*, 208 (1954) 785.
[6] M. M. WEBER, H. M. LENHOFF AND N. O. KAPLAN, *Biochim. Biophys. Acta*, 14 (1954) 298.
[7] W. JOKLIK, *Australian J. Sci. Research, Ser. B*, 3 (1950) 28.

Received August 31st, 1955

The role of nucleoside phosphorylase in erythrocyte preservation*

Erythrocytes stored in acid-citrate-dextrose (ACD) at 4° undergo a progressive loss of physiological viability, as measured by post-transfusion survival, and a simultaneous decline in the level of cellular phosphate esters, principally adenosine triphosphate and 2,3-diphosphoglycerate. Previous studies from this laboratory have demonstrated that after deterioration upon storage, cells may be rejuvenated by incubation with certain purine nucleosides[1,2]. The rejuvenation is indicated by a marked resynthesis of phosphate esters from orthophosphate (*cf.* Column I, Table I), and a concomitant restoration of the viability of the red cell**[3]. The ability of nucleosides to effect the resynthesis of phosphate esters is retained in hemolysates (*cf.* Column II) with the same order activity for the nucleosides: inosine > adenosine > guanosine > xanthosine. Deoxyadenosine and 2,6-diaminopurine riboside (kindly supplied by Dr. G. B. BROWN) were somewhat less effective than xanthosine in the intact cell system, whereas adenosine mono- and tri-sulfate (kindly supplied by Prof. Dr. A. HOCK) were inactive. Pyrimidine nucleosides (thymidine, uridine and cytidine) are inactive and do not inhibit the action of purine nucleosides. Purine bases and ribose, alone or in combination, are inactive. Trichloracetic acid filtrates of stored cells, which have been rejuvenated by the above nucleosides, contain quantities of the corresponding purine bases as revealed by paper chromatography in *n*-butanol/water (85/15). The above evidence suggests that red cell rejuvenation is initiated by the action of a purine nucleoside phosphorylase[5]:

$$\text{Purine-ribose} + P_i \longrightarrow \text{purine} + \text{ribose-1-P} \qquad (1)$$

which cleaves the nucleoside with the simultaneous conversion of orthophosphate (P_i) to ribose-1-phosphate. Subsequent metabolism of the phosphorylated pentose then provides energy for the resynthesis of phosphate esters.

TABLE I

RESYNTHESIS OF PHOSPHATE ESTERS BY NUCLEOSIDES

	*μM of organic P/100 ml red cells**	
	*Intact cells***	*Hemolysate****
Control§	400	229
Inosine	1305	917
Adenosine	1135	876
Guanosine	980	836
Xanthosine	894	687

* Phosphate measurements as described previously[4].

** Human blood, stored 25 days. 1300 μM nucleoside/100 ml cells; incubated for 45 min at 37°.

*** Hemolysate prepared from human blood stored 21 days. Values recalculated on the basis of 100 ml of cells. 1100 μM nucleoside/100 ml cells; incubated for 1 h at 37°.

§ Stored blood incubated in the absence of nucleoside.

* Aided by grants from the Office of the Surgeon General of the Department of the Army, the U.S. Atomic Energy Commission, and the Public Health Service.

The authors are indebted to Miss ENID NURK and Miss MARION HENNESSEY for their assistance on this problem.

** Similarly, if nucleosides are added to fresh cells in ACE there is a prolongation of the effective period of storage, *i.e.* approximately 40–50 days in contrast to the value of 21 days which has been established for ACD alone[3].

In order to test this hypothesis, hemolysates were examined for phosphorylase activity* with the above four purine nucleosides. For convenience in assaying the enzyme, phosphate was replaced by arsenate in the system, and the reaction was followed by the measurement of liberated ribose:

$$\text{purine-ribose} + \text{arsenate} \rightarrow \text{purine} + \text{ribose-1-arsenate} \xrightarrow{H_2O} \text{ribose} \quad (1)$$

TABLE II

NUCLEOSIDE PHOSPHORYLASE ACTIVITY

	μM ribose formed	
	Hemolysate	*Purified enzyme*
Inosine	6.0	6.0
Adenosine	3.9	0.0
Guanosine	2.4	3.6
Xanthosine	2.4	0.4

Reaction mixture contained 10 μM of nucleoside, 20 μM of arsenate, 100 μM of Tris buffer, pH 7.5, 0.5 ml of crude hemolysate or 72 μg of purified phosphorylase in a total volume of 3.0 ml. After incubation for 3 h at 37°, the solution was heated for 5 min at 100°, and a 1.0 ml aliquot was analyzed for ribose by a slight modification of the method of NELSON[8].

As shown in Column I of Table II, the relative order of activity of the nucleosides for enzymic phosphorolysis is exactly the same as for the rejuvenation phenomenon.

The nucleoside phosphorylase has been purified about 125-fold from hemolysates, using adsorption (pH 5.5) and elution (0.5 M phosphate buffer, pH 8.0) of the enzyme from calcium phosphate gel, selective heat denaturation (60° for 5 min) and ammonium sulfate fractionation (60% saturation)[1]. Enzymic activity can be followed by the arsenolysis reaction, or in the case of inosine, the system can be coupled with xanthine oxidase in order to oxidize hypoxanthine to uric acid[5]. The latter reaction can be followed manometrically (O_2 uptake) or spectrophotometrically (increased light absorption at 290 mμ). The Michaelis constant (K_m) for inosine with the purified enzyme is *ca.* 10^{-3} M, which agrees well with the "apparent" K_m value reported previously for nucleoside utilization in the rejuvenation process in the intact cell system[2].

The purified enzyme acts only upon inosine and guanosine (Column II, Table II), but with the same ratio of activities as observed in the crude hemolysate. The failure of adenosine and xanthosine to be utilized by the purified enzyme indicates that in hemolysates or intact cells, these nucleosides must be converted first to either inosine or guanosine. The conversion of adenosine to inosine by the adenosine deaminase in erythrocytes has been established previously[9,10]. From preliminary experiments, xanthosine appears to be converted to guanosine, but the mechanism of this reaction remains to be elucidated.

BEVERLY WESCOTT GABRIO
Departments of Medicine and Biochemistry, University of Washington, Seattle (U.S.A.) F. M. HUENNEKENS

1 B. W. GABRIO AND F. M. HUENNEKENS, *Federation Proc.*, 14 (1955) 217.
2 B. W. GABRIO, M. HENNESSEY, J. THOMASSON AND C. A. FINCH, *J. Biol. Chem.*, 215 (1955) 357.
3 B. W. GABRIO, D. M. DONOHUE AND C. A. FINCH, *J. Clin. Invest.*, Oct. 1955.
4 B. W. GABRIO AND C. A. FINCH, *J. Clin. Invest.*, 33 (1954) 242.
5 H. M. KALCKAR, *J. Biol. Chem.*, 167 (1947) 461.
6 Z. DISCHE in *Phosphorus Metabolism*, Vol. I, edited by W. D. MCELROY AND B. GLASS, Johns Hopkins Press, Baltimore, 1951, p. 171.
7 T. A. J. PRANKERD AND K. I. ALTMAN, *Biochim. Biophys. Acta*, 15 (1954) 158.
8 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.
9 B. W. GABRIO, C. A. FINCH AND F. M. HUENNEKENS, Erythrocyte Preservation: A Topic in Molecular Biochemistry. Submitted to *Blood*.
10 BO NORBERG, private communication.

Received October 1st, 1955

* The existence of a nucleoside phosphorylase in erythrocytes has been postulated previously by DISCHE[6] and by PRANKERD AND ALTMAN[7].