Preliminary Notes

Effect of inosine and adenine on adenosine triphosphate regeneration and shape transformation in long-stored erythrocytes

It has been reported by GABRIO *et al.*[1] and many others that the 2,3-diphosphoglyceric acid and ATP contents of preserved blood, which are considerably decreased during storage, are restored to some extent by incubation with inosine or adenosine, and that the ability of these cells to survive in the circulation after transfusion is also improved. After storage for much longer periods, however, the ATP level is not increased any longer by addition of inosine alone. This may be attributed to the exhaustion of the adenine moiety needed for the resynthesis of ATP inside the erythrocytes[2,3]. When added separately, neither adenine, adenosine, AMP or ADP is effective. However, the following experiments show that there is a marked elevation of the ATP level of the red cells if adenine is added together with inosine.

Human blood stored at 4° in acid-citrate-dextrose solution for 8 weeks was incubated at 37° for 3 h with or without addition of adenine and inosine. At the end of incubation, the blood samples were deproteinized by addition of 0.6 *N* $HClO_4$, and the nucleotides and the organic phosphate esters were analyzed by ion-exchange chromatography on Dowex-1 (formate form) employing the elution techniques of HURLBERT *et al.*[4].

TABLE I

REGENERATION OF 2,3-DIPHOSPHOGLYCERIC ACID AND ATP ON INCUBATION WITH INOSINE AND ADENINE OF BLOOD WHICH HAD BEEN PRESERVED FOR 8 WEEKS.

Incubation: 3 h at 37°. All values are expressed as μmoles/100 ml blood. I, without addition of inosine or adenine; II, with addition of inosine (25 m*M*) alone; III, with addition of inosine (35 m*M*) and adenine (20 m*M*).

Phosphorus compound	*I*	*II*	*III*
AMP	—	—	1.9
IMP	4.7	15	6.8
ADP	—	0.4	7.2
ATP	1.5	2.5	27.4
2,3-diphosphoglyceric acid	1.5	74	43.5
HDP fraction*	20.5	44	7.0
HMP fraction*	8.5	70	35

* Expressed as P.

Typical results are presented in Table I. In the samples containing added inosine but not adenine, 2,3-diphosphoglyceric acid was increased considerably, whereas the increase in ATP was not significant. In the blood incubated with both inosine and adenine the ATP level was increased up to nearly 70 % of that in fresh blood.

Abbreviations: AMP, ADP, ATP adenosine mono- di- and triphosphate; IMP, inosine monophosphate; HDP, hexose diphosphate; HMP, hexose monophosphate.

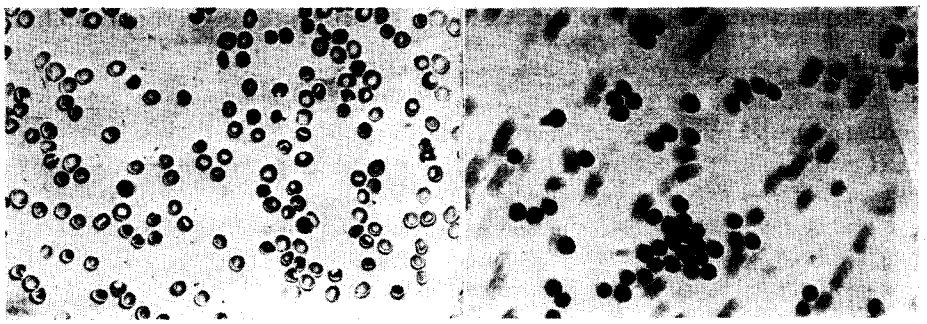

Fig. 1. Microphotographs of erythrocytes which had been stored 8 weeks and were then incubated 3 h at 37° with (A) and without (B) addition of inosine and adenine.

These findings suggest that adenine is necessary as the source of the adenine moiety for the resynthesis of ATP, while the role of added inosine is to provide the ribose residue of the nucleotide and the glycolytic intermediates necessary for the supply of phosphate.

On examination with the microscope, an interesting observation was made as to the shape transformation of the aged blood cells during incubation with adenine and inosine. At the end of 8-weeks storage, the cells were spherical, their diameter ranging between 5 and 6μ (Fig. 1, B). On incubation with added inosine and adenine, almost all of the erythrocytes were transformed to a shallow bowl form, being concave on one side (Fig. 1, A). This change of shape took place more than 30 min after addition of inosine and adenine at 37°. The diameter of the red cells was increased to about 8μ, but the hematocrit value of the blood remained almost unchanged, indicating that the shape transformation was not accompanied by a volume change of the cells.

The addition of inosine and adenine in concentrations over a wide range were effective, while separate additions of inosine or adenine were ineffective in changing the shape of aged blood cells. The intimate relationship between the restoration of the ATP level and the shape transformation of aged blood cells has also been shown in a tracer experiment using ^{32}P, which will be reported elswhere.

The present findings suggest that ATP in the erythrocyte plays a role in maintaining the disk shape of the red cell, presumably by inducing a transformation of the protein molecule constructing the ghost.

Department of Physiological Chemistry and Nutrition, Faculty of Medicine, The University of Tokyo (Japan)
Central Clinical Laboratory, The University of Tokyo Hospital (Japan)

MAKOTO NAKAO
TOSHIKO NAKAO
MASAMITI TATIBANA
HARUHISA YOSHIKAWA
TAKESHI ABE

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