Effect of Multiple Additions of Adenine-Inosine on the Function of Stored Erythrocytes[1] (35071)

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Prolongation of the viability of red cells *in vitro* has been the goal of many investigations. Freezing of red cells is theoretically a good solution; it is not as yet practical except under special conditions. Addition of nucleotides, as supplied by adenine (1) and, preferably, of adenine and a nucleoside, inosine (2–4), has achieved considerable success. However, while searching for longevity, study of the function of stored cells has been neglected. Valtis and Kennedy reported (5) that the affinity of hemoglobin for oxygen increases with storage of blood in acid–citrate–dextrose (ACD) solution in the cold. With this increased affinity, oxygen release to tissue is decreased; several hours are required for partial restoration of normal oxygen release. Such delay may have serious consequences in the case of acute blood loss. In 1967, Benesh and Benesh (6) and Chanutin and Curnish (7) found that certain phosphorylated compounds, particularly adenosine triphosphate (ATP) and 2, 3-diphosphoglycerate (2,3-DPG) control the oxygen affinity of hemoglobin; ATP and 2, 3-DPG appear to have a similar effect on a molar basis (8).

Adenine added to ACD blood, while increasing the useful period of storage to 35 days, was found to increase the oxygen–hemoglobin affinity (9); incubation of stored ACD blood with inosine at 37—° was found to restore partially ATP, 2,3-DPG, and oxygen dissociation (8, 9). Bunn *et al.* (9) state that when inosine was added to ACD-adenine blood at the time of collection, oxygen affinity increased much more slowly; addition of inosine at 20 days of storage and incubation at 37°, showed improvement of the level of 2,3-DGP and ATP, with decreased oxygen affinity. Valeri and Hirsh (10) found that the ATP of stored ACD cells increases rapidly after transfusion, but 2, 3-DPG increases more slowly, being above the 50% level at 24 hr postransfusion and requiring 11 days to reach the maximal level. The improvement of the oxygen dissociation obtained by addition of inosine is of relatively short duration (8, 9); when inosine is added to red cells stored for a period of several weeks the improvement is small.

Previous studies (4) had shown that multiple additions of adenine–inosine to stored blood increased significantly the ATP level and extended the viability of red cells to 56 days of storage, (73.3% survival, 24 hr posttransfusion with a normal T-½). These results suggested the study of the effect of multiple additions of adenine and inosine on the 2,3-DPG and oxygen dissociation of red cells of ACD blood.

Procedure and Methods. Blood was collected from nine young healthy individuals into plastic bags containing acid–citrate–dextrose solution (ACD) NIH formula A.[2] Samples for 0-time determinations were obtained immediately after collection. The ACD blood was then chilled in chipped ice and divided into a number of aliquots of 10- to 50-ml vol, according to the nature of the study. These were maintained undisturbed at 1° ± 0.5 until the time of use.

Adenine and inosine (AI) were combined

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in a solution containing: Adenine base 0.047 g; inosine 1.400 g; parenteral 0.9% saline solution 100 ml. Of this solution 0.6 ml were used per gram of hemoglobin to obtain a concentration of adenine and inosine of 2 and 30 μmoles per gram of hemoglobin, respectively. Sterilization was accomplished by passage through a Millipore filter with pores of 0.22 μ. Regeneration was carried out at 1° for 1 or more days. The determination of the oxygen–hemoglobin equilibrium has been done with the method described by Edwards and Martin (11), using the micro-equilibration apparatus developed by Siggaard-Andersen *et al.* (12). Oxygen dissociation was measured at 50% saturation point and will be expressed as P_{50} in millimeters of mercury. The estimation of 2,3-diphosphoglycerate was carried out by the procedure of Beutler *et al.* (13); the adenosine triphosphate content by the method of Beutler and Mathai (14).

Results. Control values in freshly collected ACD blood from young healthy subjects are shown in Table I. Additions of adenine and inosine (AI) were made to blood stored at 1°. Results shown in Fig. 1 are expressed in percentage of original values for purpose of comparison. The number of additions and the interval between additions was varied, as follows: six additions, at 0, 2, 4, 7, 11, and 16 days of storage; four additions, at 0, 2, 4, and 7 days; three additions, at 0, 2, and 4 days; three single additions, at 0, 7, and 14 days of storage. After the first week, the ATP of blood stored in plain ACD solution drops fairly linearly and slowly so that at 21 days the level is close to 80% of the original value. The 2,3-DPG drops rapidly, being down to about 40% at 7 days of storage and less than 5% at 21 days. The P_{50} drops rapidly during the first week of storage to about 70% of the original, but after that it drops slowly, the value being 66% of the original at 21 days. Therefore, there is a good correlation between 2,3-DPG and the P_{50} for the first week but after that the correlation is not so close. There is a good correlation between the sum of molar concentrations of ATP and 2,3-DPG and the P_{50} ($r = 0.6672$ at 1% level). Optimal preservation of ATP, 2,3-DPG and P_{50} were obtained with

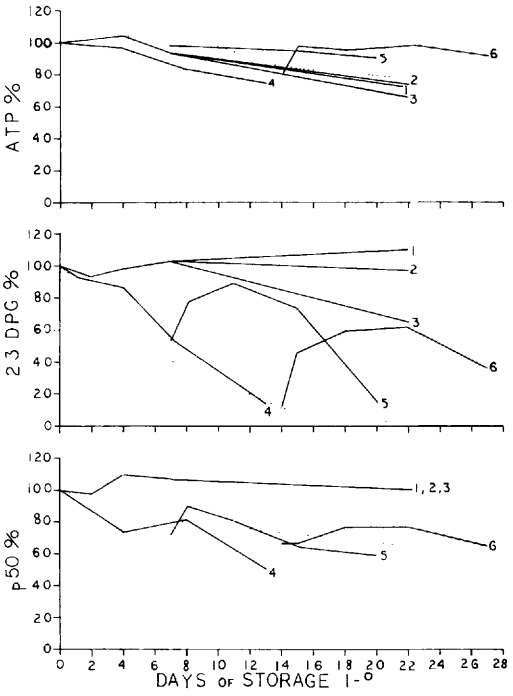

FIG. 1. Each value represents the mean of determinations on three separate units of blood. Number at the end of each plotted line refers to experiments listed as follows: (1) six additions of adenine-inosine, at 0, 2, 4, 7, 11, and 16 days of storage; (2) four additions at 0, 2, 4, and 7 days; (3) three additions at 0, 2, and 4 days; (4) single addition at 0 time; (5) single addition at 7 days; (6) single addition at 14 days. The dotted lines show control values obtained with plain ACD stored blood.

TABLE I. Biological Variations and Reproducibility of Tests in Freshly Collected ACD Blood from Young, Healthy Subjects.

	ATP[a]	2,3-DPG[a]	P_{50}[b]
Biological variations			
Range	3.5–6.0	8.4–18.0	21.2–32.8
Mean	4.49	13.90	26.90
S.D.	0.81	2.97	2.90
C.V.	18.1%	21.0%	11.0%
Reproducibility			
C.V.	3.6%	5.0%	2.0%

[a] ATP and 2,3-DPG are expressed as micromoles/g hemoglobin.

[b] P_{50} as mm of mercury at 50% saturation.

six additions of AI representing a total of 12 μmoles of adenine and 180 μmoles of inosine per gram of hemoglobin; similar results were obtained with four additions of AI representing 8 μmoles of adenine and 120 μmoles of inosine per gram of hemoglobin, the last addition occurring on day 7 of storage.

Statistically (chi-square test), multiple additions of AI produce a very significant increase of 2,3-DPG when compared with values obtained in plain ACD blood at 22 days of storage (three additions vs control: 43.1673; four additions: 62.2727; six additions: 73.5007). Single additions of AI generally show no sustained increase of 2,3-DPG. Addition at 14 days shows a substantial increase of ATP; in another experiment the first addition of AI was made at 13 days and weekly thereafter for 10 weeks. The 2,3-DPG after day 28 of storage dropped to negligible quantities, but ATP was maintained, being 75% of the original value at 70 days of storage.

Discussion. Garby and deVerdier reported (15) that under near physiological conditions, considerable amounts of ATP and 2,3-DPG were bound to hemoglobin, thus permitting a normal release of oxygen to tissues. We have shown that ATP, 2,3-DPG and P_{50} of ACD-stored red cells can be maintained at normal levels for at least 22 days of storage at 1° by multiple additions of adenine and inosine. Considering the slow utilization of adenine, it is evident that for a period of 28–35 days of storage of blood in ACD solution, a single initial dose of adenine should be sufficient to maintain an adequate amount of ATP, hence of red cell viability (1). Previous experience indicates that an addition of 2 μmoles of adenine per gram of hemoglobin with 20–30 μmoles of inosine is sufficient to maintain viability of red cells for at least 35 days, on the basis of 70% survival, or better, 24 hours posttransfusion (4). Inosine on the other hand is rapidly utilized and a single addition to fresh blood to obtain a concentration of 30 μmoles per gram of hemoglobin exerts a beneficial effect on the 2,3-DPG for less than 7 days. When four or six cumulative doses of AI are added to blood beginning at 0 time, at a rate exceeding the capacity of the red cell to utilize inosine, the 2,3-DPG, ATP, and P_{50} are maintained at the level found in fresh blood for at least 22 days; with four additions, from 0–7 days, no further additions are required for 2 weeks. Very frequent additions, therefore, are not needed but it is evident that when the first addition is made after day 13 of storage there is no significant restoration of 2,3-DPG or P_{50}. It is also apparent that multiple additions of adenine, in excess of the requirement, failed to alter the effect of inosine when inosine was added in sufficient amounts. Accumulation of hypoxanthine from large or repeated doses of inosine should not exclude the use of blood thus treated for clinical purposes, because with a single washing of red cells with saline, hypoxanthine and extracellular unmetabolized adenine and inosine are reduced to 4% of the original concentration.

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