A STUDY OF STORAGE LESIONS IN CORD AND ADULT BLOOD

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ABSTRACT

A STUDY OF STORAGE LESIONS IN CORD AND ADULT BLOOD

Cord and adult blood was stored at $4^{\circ}C$ in a citric acid-sodium citrate-dextrose medium. It was observed that in the first three days of storage changes in intracellular cation concentrations were more marked in cord blood. Results indicated that this could not be due to differences in the hemoglobins of the two kinds of blood.

The storage of cord and adult blood with adenosine was found to result in an increase of intracellular sodium, a decrease in intracellular potassium and a decrease in erythrocyte volume. The possible cause of these effects is discussed.

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PART A

INTRODUCTION

THE METABOLISM OF THE ERYTHROCYTE

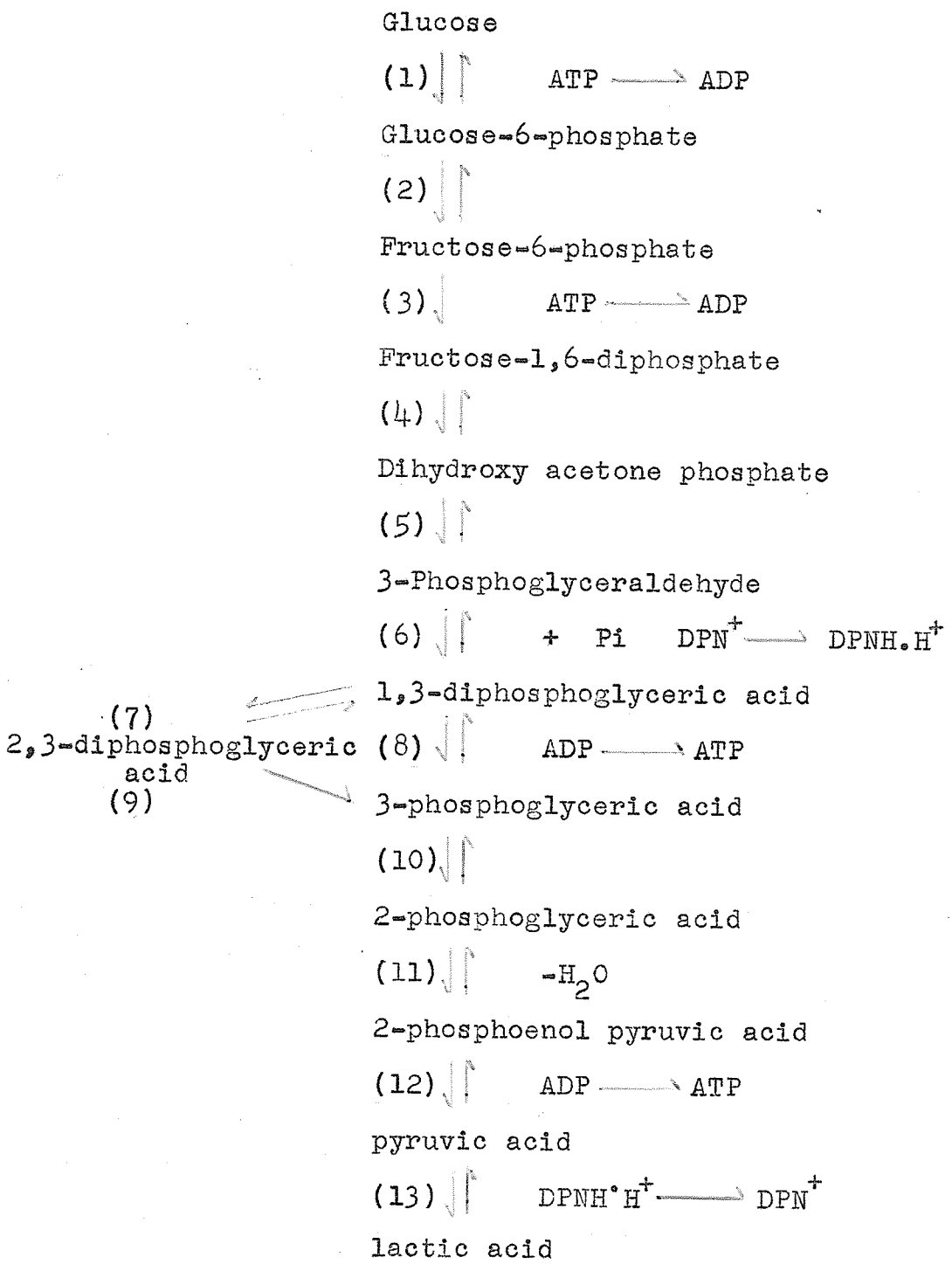
The human erythrocyte, long thought of as merely a container for hemoglobin, is now known to be a complex metabolizing cell with a wide variety of enzyme systems. The erythrocyte maintains a high intracellular potassium and a low intracellular sodium concentration against concentration gradients. Glucose and inorganic phosphate are actively transported across the cell membrane from the plasma. The cell maintains the concentration of methaemoglobin at a low level and preserves its shape and integrity for approximately 120 days in vivo.

In normal subjects the cell obtains the energy for these functions from the anaerobic conversion of glucose to lactic or pyruvic acids via a series of phosphate esters of the glycolytic pathway. The depletion of glucose, or the inhibition of its metabolism by enzyme poisons results in a fall of intracellular potassium, a rise in intracellular sodium, an increase in methaemoglobin concentration, an alteration in cell shape and finally cell lysis.

<u>Glycolysis:</u> In the erythrocyte, glucose is broken down through the phosphorylated intermediates of the

FIGURE I

THE GLYCOLYTIC PATHWAY



ENZYMES OF THE GLYCOLYTIC PATHWAY

1. Glucokinase or hexokinase
2. Phosphohexose isomerase
3. Phosphohexokinase
4. Aldolase
5. Phosphotriose isomerase
6. 3 - Phosphoglyceric aldehyde dehydrogenase
7. Diphosphoglyceric acid mutase
8. Phosphoglyceric kinase
9. Phosphatase
10. Phosphoglyceromutase
11. Enolase
12. Pyruvic phosphokinase
13. Lactic acid dehydrogenase

Embden-Meyerhof-Parnas-Cori pathway (Figure I). The red cell is unique in its 2,3-diphosphoglycerate concentration. This compound is a co-enzyme for phosphoglyceromutase, and is found in trace amounts in most tissues; in the red cell however approximately one half of the organic phosphate is in this form. Its function is unknown although it may exist as a store of energy as does creatine phosphate in muscle.

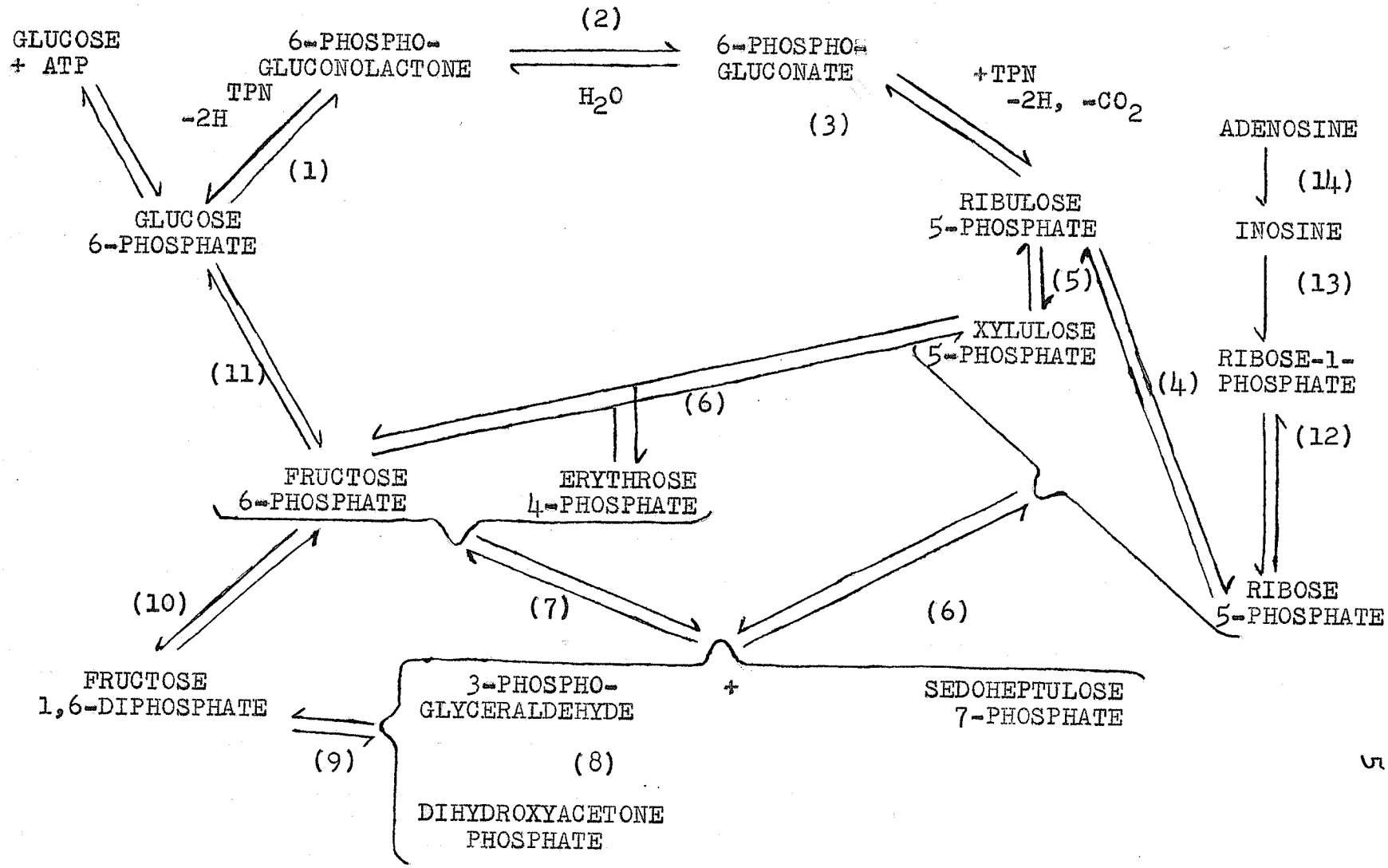
The citric acid cycle is not active in the erythrocyte, although some of its enzymes are present.

Glucose is not the only substrate that erythrocytes can utilize. Bevilotti (1944) found that the red cells of patients with malignant tumors decomposed pentoses aerobically to lactic acid. Dische (1951) found that hemolysates converted ribose phosphates, or the ribose moiety of nucleosides, to hexose and triosephosphates. Prankerd and Altman (1954), and Gabrio, Donahue and Finch (1955) found that adenosine, guanosine and inosine caused organic phosphate synthesis and stimulated phosphate uptake even in the absence of glucose.

Adenosine is deaminated by the red cell to inosine (Rubinstein, Kashket and Denstedt - 1956). In the presence of inorganic phosphate, the nucleoside is phosphorylated and split to hypoxanthine or guanine by

FIGURE II

THE HEXOSE MONOPHOSPHATE SHUNT



ENZYMES OF THE HEXOSE MONOPHOSPHATE SHUNT

1. Glucose-6-phosphate dehydrogenase (Zwischenferment)
2. Lactonase
3. Phosphogluconic dehydrogenase
4. Phosphoriboisomerase
5. Phosphoketoepimerase
6. Transketolase
7. Transaldolase
8. Triosephosphate isomerase
9. Aldolase
10. Fructose diphosphatase
11. Hexose phosphate isomerase
12. Ribose phosphate isomerase
13. Nucleoside phosphorylase
14. Adenosine deaminase

purine nucleoside phosphorylase (Huennekens, Nurk and Gabrio - 1956). The ribose-1-phosphate moiety is converted to triose or hexose phosphates via the hexose monophosphate shunt (Figure II).

In the presence of methylene blue, erythorcytes aerobically oxidize glucose. Brin and Yonemoto (1958) have suggested that it is so oxidized via the hexose monophosphate shunt. It is doubtful if the shunt has any physiological importance in the mature erythrocyte; it is likely that some erythrocyte precursor utilizes the shunt in its aerobic metabolic system.

Ion Transport: The concentration gradients which exist between intracellular and extracellular sodium and potassium are not maintained by membrane impermeability but by an active transport mechanism which creates a flux of ions into and out of the cell to counterbalance a passive diffusion along the concentration gradient.

The active transport of ions is linked to glycolysis and the formation of ATP. Active transport ceases if glycolysis is inhibited. ATP is almost certainly involved in ion transport since strophanthin, which blocks active ion transport, also reduces ATP loss in incubated erythrocytes (Dunham, 1957).

The active transport of sodium and potassium is directly linked. Post and Jolly (1957) found that the absence of external potassium or absence of internal sodium would stop the active transport of both ions. They suggested that two atoms of potassium are transported for every three atoms of sodium, while Harris (1957) concluded that the ratio is 1:2.

Some workers (Solomon - 1952, Solomon and Gold-1954) suggest that the ions are transported by a carrier, and Kirschner (1957) has found a compound, phosphatidylserine, in red cells which distinguishes between sodium and potassium and forms a lipid-soluble compound with sodium.

Phosphate uptake by the red blood cell, as measured by P^{32} uptake is an active process linked to glycolysis by conversion of 3-phosphoglyceraldehyde to 1,3 diphosphoglycerate at the cell surface (Prankerd and Altman - 1954). Orthophosphate leaves the cell by slow, passive diffusion.

THE LESION OF STORAGE

When blood is stored under varying conditions, the red cells shange in several ways. When blood is stored at $4^{\circ}C$ in citric acid-sodium citrate-dextrose

solution (ACD), there is a progressive hydrolysis of intracellular phosphate esters, accompanied by a loss of inorganic phosphate into the plasma. During storage, the red cells lose potassium, gain sodium, the rate of glycolysis decreases, the red cell becomes crenated, its osmotic fragility increases, and its *in vivo* survival after transfusion is shortened. Gabrio, Stevens and Finch (1954b) and Crawford and Mollison (1955) found that these changes are reversed in these cells *in vivo* following transfusion.

Gabrio, Stevens and Finch (1954a) termed the changes in red cell metabolism during storage the *lesions of storage*. They eliminated plasma, leucocytes, reticulocytes, hemolysates, citrate and the gas phase as contributing or alleviating factors in this lesion (1954a), and showed that these changes were not related to the *in vivo* age of the cell (1954). They concluded that the lesions reflected a derangement in the carbohydrate metabolism of the red cell, occurring under storage conditions.

Gibson, Murphy, Scheitlin and Rees (1956) found that *in vivo* survival was shorter in the first cells collected into ACD. They pointed out that these cells are suddenly placed in a hypotonic medium with a lower

pH and lower temperature than exists in the blood *in vivo*. Collection is accompanied by foaming and turbulence, and cells are damaged by contact with the "wetting" surfaces of the container. The resulting damage to the cells they called the *lesion of collection.*

Gabrio *et al* (1955) found that the addition of the nucleosides, adenosine, inosine, or guanosine to stored blood increased the viability of the cells, restored intracellular organic phosphate levels, partly reversed potassium loss and decreased osmotic fragility. They suggested that the nuceloside was phosphorylated and split at the cell wall to form ribose-1-phosphate, which was converted to triosephosphate through part of the hexose monophosphate shunt, as shown in Figure II.

The in vitro reversal of storage lesions by nucleosides suggests that the initial failure in metabolism during storage occurs during the conversion of glucose to triosephosphate, since the lesion can be prevented or reversed by the introduction of a metabolite at the triosephosphate level. Brownstone and Blanchaer (1957) suggested that the integrity of phosphofructokinase may influence the keeping qualities of ACD - preserved blood, and found that adenosine maintained the activity

of this enzyme at a normal level. Gabrio, Hennessey, Thomasson and Finch (1955) have suggested that a decrease in ATP causes a decreased velocity in the first step of glycolysis, namely the phosphorylation of glucose by ATP. Jorgensen and Chen (1956, 1957) have proposed that cell damage during collection activates a dephosphorylation of ATP, thereby slowing the phosphorylation of glucose.

HEMOGLOBIN AND RED CELL METABOLISM

The major component of red cells is hemoglobin, which constitutes over 90% of the cell's dry weight. The hemoglobin of red cells is predominantly "adult" hemoglobin - Hb A. However, some human bloods contain other hemoglobins, and some of these are associated with abnormal cell structure or metabolism.

In sickle cell anaemia, much of the hemoglobin is Hb S. Bartlett, Hughes, Barney and Marlow (1955) failed to find any abnormality in glucose utilization or phosphate uptake in sickle cells. Sodium and potassium fluxes were normal. However, when the hemoglobin is de-oxygenated, the cell is distorted, cell potassium loss is rapid (Tosteson, 1953) and cell sodium increases

(Clarkson and Maizels, 1955). Cells containing Hb S, and those containing another hemoglobin, Hb C, have short survival times when transfused into normal recipients (Sprague and Paterson, 1956).

The red cells of the newborn contain varying amounts of "fetal" hemoglobin (Hb F), as do the cells of people with thalassemia. Dervichian, Fournet, Guinier and Ponder (1952) found that the red cells of the newborn and of thalassemia major and minor have abnormal ultrastructures, suggesting a different association of hemoglobin with the lipo-proteins of the cell wall. De Luca and Caruso (1957) found a lowered resistance to saponin in red cells containing Hb F. As measured by the radio-chromium technique, the cells of the newborn and those of thalassemia both have slightly shortened survival times when transfused to normal subjects, (Hollingsworth, 1955; Hamilton, 1950). This may be due, however, to the more rapid elution from Hb F of the Cr^{51} (Suderman, White and Israels, 1957).

Israels, Chutorian, Delory and Israels (1958) found that the formation of sulfhemoglobin shortened the life span of rabbit erythrocytes. These results, and the association of abnormal hemoglobins with defective red cells, led to the suggestion that hemoglobin might

play some role in the metabolism of the red cell.

SCOPE OF THE THESIS

Apart from the growing importance of blood storage and transfusions in medicine, the study of storage lesions provides an insight into the metabolism of the erythrocyte. Since cord blood (x) differs markedly from adult blood in many respects, it seemed of interest to study its behaviour during storage. This was to obtain information about the lesion of storage as well as to provide a means of observing if the presence of Hb F affects red cell metabolism.

The first part of this work consisted of a study of the storage of cord blood and a comparison of storage lesions in adult and cord blood. The results observed in this work raised some further questions relating to cord erythrocyte metabolism. The effect of adenosine on cord blood storage, as well as a comparison of the effect of adenosine on cord blood and adult blood was then undertaken in an attempt to explain some of the initial findings.

(x) Note: The terms fetal blood, cord blood, placental blood and blood of the newborn are used interchangeably in the literature. The term cord blood will be used throughout the thesis.

PART B

ANALYTICAL METHODS

INTRACELLULAR SODIUM AND POTASSIUM CONCENTRATION

Reference: A. Zipursky, personal communication (1957).

Reagents: 1. De-ionized water. Water was twice distilled, then passed through Fischer Amberlite MB-3 ion exchange resin. The water and reagents were stored in pyrex or polyethylene vessels.

2. Non-Ionic Detergent. One gm. Sterox CD or Sterox SE (Monsanto Chemical Co.) in 100 mls. de-ionized water.

3. Stock KCL solution - 10 meq./KCL. .7455 gm. dry KCL in one liter de-ionized water.

4. Stock NaCl solution - 250 meq./NaCl. 7.306 gm. dry NaCl in one-half liter de-ionized water.

5. Standard solutions were prepared from the above reagents.

Procedure: Blood was centrifuged at 800 gravities for fifteen minutes. The plasma was removed for sodium and potassium analysis and the red cells transferred by

pipette to a glass tube 12 cm. long and of 3 mm. internal bore. This tube was centrifuged at 1200 gravities for sixty minutes. Following centrifugation the tube was broken about one cm. below the top of the red cell mass. The contents of the larger part of the tube were washed with de-ionized water into a 10 ml. volumetric flask, using a long, U-shaped #19 needle and a 5 ml. syringe.

The volumetric flask was filled to the mark with de-ionized water, and aliquots taken for sodium and potassium analysis. The dried tube was filled from a burette to determine the volume of red cells used. Intracellular sodium and potassium determinations were calculated assuming 2 per cent plasma trapping (Chaplin and Mollison, 1952.)

Ion concentrations were obtained using a Coleman Model 21 Flame Photometer fitted with a Model 22 Galvanometer, following the manufacturer's instructions. For potassium, standards were prepared for the ranges 0 - 10, 0 - 50 and 0 - 100 meq./liter. Since with this apparatus potassium increases the apparent sodium value, the procedure was standardized for the range 0 - 175 meq./l Na, with potassium backgrounds of 0, 50, 100, 150 and 200 meq./l K, and experimental values were obtained by interpolation.

Reproducibility of the Procedure: Adult blood which had been stored at 4°C in ACD for two weeks was mixed to resuspend the cells. Blood was poured into ten test tubes, and intracellular sodium and potassium concentrations determined on each sample. Five determinations of sodium and potassium were determined on the whole blood.

Results:

No. of Samples	Analyzed for	Mean Value meq./l	Standard Deviation
5	Whole Blood K	37.8	.55 (1.5%)
5	Whole Blood Na	96	2.7 (2.8%)
10	RBC K	67.6	1.95 (2.9%)
10	RBC Na	37.9	2.3 (6.2%)

PHOSPHATE FRACTIONS

Reference: Gomori, G. : J. Lab. Clin. Med. 27, 955, 1942.

Fiske, C.H. and Subbarow, Y.: J. Biol. Chem. 66, 375, 1925.

Reagents:

1) 10 N H_2SO_4
2) 1 N H_2SO_4
3) Elon solution: One gm. methyl-p-amino phenol sulphate in 100 mls. 3% (w/v) sodium bisulfite.

4) 5% (w/v) Na_2MoO_4 in water

5) Molybdic acid: Two parts Na_2MoO_4 solution, one part 10 N H_2SO_4, one part water

6) Trichloroacetic acid (TCA): 100 gms./liter

Procedure: .5 or 1 ml. of sample was flushed into a tube containing 2 ml. of H_2O. Three ml. of TCA were added, and the mixture filtered through Whatman #44 filter paper into a 10 ml. volumetric flask. The tube and paper were rinsed with a further 5 ml. of TCA, and the filtrate diluted to 10 ml. Filtrates were frozen till used.

Inorganic Phosphate (IP) An aliquot of the filtrate was placed in a 10 ml. volumetric flask, and 2.5 ml. of mobydic acid solution and 1 ml. of elon solution added. The volume was made up to 10 ml. and the concentration determined colorimetrically at 610 mu with a Coleman Junior Spectrophotometer 6A.

Total Acid Soluble Phosphate (TASP) One ml. of filtrate was heated with 1.5 ml. of 10 N H_2SO_4 until dense fumes ceased coming off. H_2O_2 was added to discharge the brown color, and the flask heated again until boiling

stopped. Molybdic acid and elon were added as above and the concentration determined.

Easily Hydrolyzable Phosphate (EH) This fraction includes ATP and ADP, and is determined as the increase in inorganic phosphate after 1 ml. of TCA filtrate is heated 7 minutes at 100°C with 3 mls. of 1 N H_2SO_4.

Difficulty Hydrolyzable Phosphate (DH) This fraction, composed mainly of AMP and hexose phosphates, is measured as the increase in inorganic phosphate from 7 to 100 minutes at 100°C in 1 N H_2SO_4.

Non-Hydrolyzable Phosphate (NH) This fraction, mainly 2, 3 diphosphoglyceric acid (2,3-DPG) is the portion of TASP not hydrolyzed by heating the TCA filtrate 100 minutes at 100°C in 1 N H_2SO_4.

Reproducibility of the Procedure: Five determinations were made of the TASP, IP, 7 minute and 100 minute fractions of a sample of blood.

	Concentration (mgms. P/100 cc)	Standard Deviation
Inorganic Phosphate	4.80	.25 (5%)
7-minute fraction	7.47	.43 (6%)
100 minute fraction	12.63	1.04 (8%)
TASP	23.28	.98 (4%)

GLUCOSE

<u>Reference:</u> Nelson, N. J. Biol. Chem. <u>153</u>, 375 (1944)

Somogyi, M. J. Biol. Chem. <u>160</u>, 69 (1945)

<u>Reagents:</u>

1) 5% (w/v) $ZnSO_4 \cdot 7H_2O$

2) .3N $Ba(OH)_2$

3) Nelson's Reagent

   25 gm. $(NH_4)_2$ in 450 ml. H_2O

   21 ml. conc. H_2SO_4

   3 gm. $Na_2AsO_4 \cdot 7H_2O$ in 25 ml. H_2O

   The mixture is incubated at 37°C for 48 hours and filtered.

4) Somogyi's Reagent

   28 gm. Na_2HPO_4

   100 ml. IN NaOH

   40 gm. Rochelle salt

   8 gm. $CaSO_4$

   180 gm. Na_2SO_4

   The mixture is diluted to one liter and filtered.

Procedure: 0.1 ml. of blood was flushed into 3 ml. of H_2O. 0.2 ml. of $Ba(OH)_2$ were added, followed by 0.2 ml. $ZnSO_4$. The solution was filtered into a 25 ml. Folin-Wu tube. Two ml. of Somogyi's reagent were added, the mixture was placed in boiling water for 10 minutes, then cooled rapidly. One ml. of Nelson's reagent was added, the mixture diluted to 25 ml. and the concentration determined colorimetrically at 500 or 660 mu.

Reproducibility of the Procedure: Ten determinations were carried out on a sample stored in ACD. The mean value obtained was 247 mgms./100 ml. The standard deviation was 11 mgms./100 ml., or 4%.

HEMOGLOBIN

0.1 ml. of blood was diluted to 25 ml. with 0.1% Na_2CO_3. The concentration was determined as oxyhemoglobin colorimetrically at 540 mu, using a Coleman 6A Spectrophotometer.

pH

pH was measured with a Beckmann glass electrode pH meter. This instrument is accurate to .01 pH units.

HEMATOCRIT

Hematocrits were measured with an International Co. high speed micro-hematocrit centrifuge. The standard deviation of twenty determinations was .725%.

MEAN CORPUSCULAR VOLUME

Red cell counts were obtained by direct microscopic counting in an erythrocyte chamber. Mean corpuscular volume was determined as:

$$MCV = \frac{\text{volume of packed cells in 1000 ml.}}{\text{red cells in millions per cu. mm.}}$$

The mean error of the procedure is 10%.

ALKALI RESISTANT HEMOGLOBIN

Reference: White, F. D., Delory, G. E. and Israels, L. G.: Can. J. Res. 28, 231, 1950.

Procedure: A hemolysate was diluted with 1% Na_2CO_3 until it had an optical density of .9 - 1.1 as measured with a Beckman DU spectrophotometer at 578 mu.

The optical density of the following solutions were measured:

A: Three mls. hemolysate + 0.6 mls. H_2O

B: Three mls. hemolysate + 0.6 mls. .25N NaOH, measured at two minute intervals.

C: Three mls. hemolysate + 0.6 mls. .25N NaOH, measured after 24 hours.

A graph was plotted of 2 log $\frac{B - C}{A - C}$ against time (minutes). The plot of the denaturation of the alkali resistant fraction was extrapolated to time = o, and the anti-log of the intercept yielded the Hb F as a per cent of total hemoglobin. The reproducibility of the procedure is 2%.

PART C

THE STORAGE OF CORD AND ADULT BLOOD

INTRODUCTION

Red cell storage: The value of transfused blood lies chiefly in the ability of the erythrocytes to perform their gas carrying role in the circulation of the recipient. Studies in blood preservation are therefore directed primarily towards the optimal preservation of the erythrocytes. The survival of these cells in the recipient is measured by agglutination techniques or the more recent radio-chromium method.

The earliest *in vitro* criterion of red cell survival was the occurrence of hemolysis. Subsequently plasma potassium and inorganic phosphate concentrations were measured. Most recently the *in vitro* tests of satisfactory red cell storage have been the intracellular sodium and potassium concentrations, the partition of phosphate esters and the ability of the cell to metabolize glucose.

The storage medium: It was noted by Rous and Turner in 1916 that the addition of glucose to citrated blood retarded hemolysis. Later it was observed by Maizels and Wittaker (1940), Mollison and Young (1942) and Loutit, Mollison and Young (1943) that lowering the

pH of stored blood increased its survival, and the latter authors proposed the citric acid - sodium citrate-dextrose (ACD) preservative solution in common use today.

Characteristics of cord blood: Apart from its hemoglobin, cord blood differs in several respects from adult blood. Plasma sodium and potassium concentrations are higher in cord blood (Gyllensward and Josephson, 1957). The cord red cells are larger and more numerous (Wintrobe, 1946), have an increased mechanical fragility (Hsia and Goldbloom, 1954) and a different ultrastructure (Dervichian, Fournet, Guinier and Ponder, 1953). The cord red cells are less active in cholinesterase, glyoxalase, carbonic anhydrase and catalase (Jones, and McCance, 1949) and have a lower concentration of methemoglobin reductase (Lonn and Molusky, 1957).

Hollingsworth (1955) and Adner and Sjolin (1957) found that cord erythrocytes tagged with radio - chromium had a short lifespan when given to normal adult recipients. Zipursky (1958) has found that P^{32} uptake by cord red cells is less than that of adult red cells.

The storage of cord blood: The first reference to the use of cord blood for transfusion is found in an article by Rubin (1914). In 1938 Goodall, Anderson, Altman and McPhail reported a method for collecting and

storing cord blood with sodium citrate. Cord blood was a popular transfusion medium for about five years thereafter, principally because it was free from food allergens and had a high hemoglobin and red cell count. (Page, Seage and Ward 1939). During this period Rotfeld (1939) noted that plasma potassium and inorganic phosphate rose more rapidly in cord blood than in adult blood during the first five days of storage.

In 1954, Sjolin studied the behaviour of cord and adult blood during storage in glucose and heparin at 4°C and 37°C. He found that adult cells hemolysed less readily, and did not lose potassium for several hours after collection. The cord erythrocytes were more water permeable and lost potassium immediately after collection. Sjolin claimed that these differences were due to differences in the cell membranes.

Greenwalt and Ayers (1956) measured the phosphate partition of cord and adult erythrocytes after twenty-one days storage in ACD. They discovered that in the cord cells there was a more marked fall in organic phosphate concentrations. Their results indicated that there was no decrease in the TASP concentration of erythrocytes, as there was in adult cells. This decrease is caused by the outward diffusion of orthophosphate into the plasma (Rapoport,1949).

Hemoglobin during storage: Despite its important role in transfused blood, relatively little work has been done on changes in hemoglobin during storage. Donahue, Finch and Gabrio (1956) noted a small drop in oxygen capacity during storage in ACD. Gullbring and Strom (1956) discovered that the hemoglobin - oxyhemoglobin curve was displaced in blood stored twenty days in ACD. Titov (1956) observed that some choleglobin was formed after ten days storage and Berry and Chanutin (1957, 1958) found that the electrophoretic pattern of hemoglobin was changed during storage.

Object of the experiment: It was decided to compare the storage properties of cord and adult blood by measuring their intracellular sodium and potassium concentrations, plasma glucose, pH and inorganic phosphate concentrations during storage in ACD. The orthophosphate partition in cord blood was investigated to determine if Greenwalt and Ayers' observation that cord erythrocytes did not lose TASP was valid.

Since Hb F features a resistance to alkali denaturation, it was decided to investiage this characteristic in cord blood stored in ACD at 4°C.

METHODS

A comparison of cord and adult blood: Twenty ml. of cord blood was collected into an evacuated sterile

twenty-five ml. flask containing five ml. of ACD solution (Abbott Laboratories Ltd.). The blood was transferred in 3.5 ml. portions to sterile evacuated tubes. One tube was prepared for analysis within thirty minutes of collection and the remaining tubes stored at 4°C.

380 ml. of adult blood was collected into 120 ml. of ACD, and ten ml. portions transferred to evacuated sterile tubes. One tube was prepared for analysis within one hour of collection. The remaining tubes were stored undisturbed at 4°C. Tubes of adult and cord blood were taken for analysis after 3, 7, 14, and 28 days storage.

The samples were analyzed for intracellular sodium and potassium, whole blood inorganic phosphate concentration, plasma glucose and pH.

Orthophosphate partition: Three samples of cord blood were collected into ACD and stored at 4°C. The whole blood TASP, plasma TASP and plasma inorganic phosphate concentrations were determined on the day of collection and after 21 days storage. In two other samples the inorganic phosphate concentration was determined in whole blood and plasma on the day of collection

and after 7, 14 and 28 days storage. Red cell inorganic phosphate concentration was calculated by difference.

Alkali resistance of hemoglobin: Four to five ml. of cord blood were collected and transferred to each of two sterile tubes containing 1 ml. of ACD. One tube was centrifuged to sediment the red cells, the plasma was removed and the cells hemolysed by the addition of 1 volume of water. One ml. of the hemolysate was added to 100 ml. of 0.1% sodium carbonate and the alkali resistant fraction (Hb F) and the denaturation rate determined by the method of White, Delory and Israels (1950). The concentration of the alkali resistant fraction and the denaturation rate were similarily determined on the hemoglobin in the second tube after it was stored twenty-one days at 4°C. All denaturations were carried out at 27.5 ± 1°C.

RESULTS

The sodium concentration of stored erythrocytes is tabulated in Table 1A. During four weeks storage the sodium concentration rose from a mean of 12.2 ± .5 meq./l red cells to 27.6 ± 3.4 in adult blood and

TABLE 1.

SODIUM CONCENTRATION IN STORED ERYTHROCYTES

(meq./l. of red cells)

Sample		Days of Storage 0	3	7	14	21	28
Adult	1	12.1	12.5	22.7	27.2	27.5	33.0
	2	11.5	13.5	20.5	20.0	29.6	28.1
	3	12.0	15.9	13.5	20.7	31.7	27.2
	4	12.3	14.0	16.0	24.6	26.0	26.1
	5	12.9	14.8	15.0	21.0	20.0	22.5
Mean ± SD		12.2 ± .5	14.1 ± 1.0	17.7 ± 3.5	22.7 ± 3.1	26.9 ± 4.0	27.6 ± 3.4
Cord	1	11.0	18.4	22.1	25.6	29.7	31.7
	2	14.0	24.7	19.4	21.9	24.5	32.0
	3	11.0	20.5	21.9	22.3	25.4	31.0
	4	11.9	12.4	17.0	23.8	32.5	28.5
	5	11.2	16.2	22.0	27.0	27.9	29.3
Mean ± SD		11.8 ± .8	18.5 ± 4.1	20.5 ± 2.2	24.2 ± 2.2	28.0 ± 3.6	30.5 ± 1.6

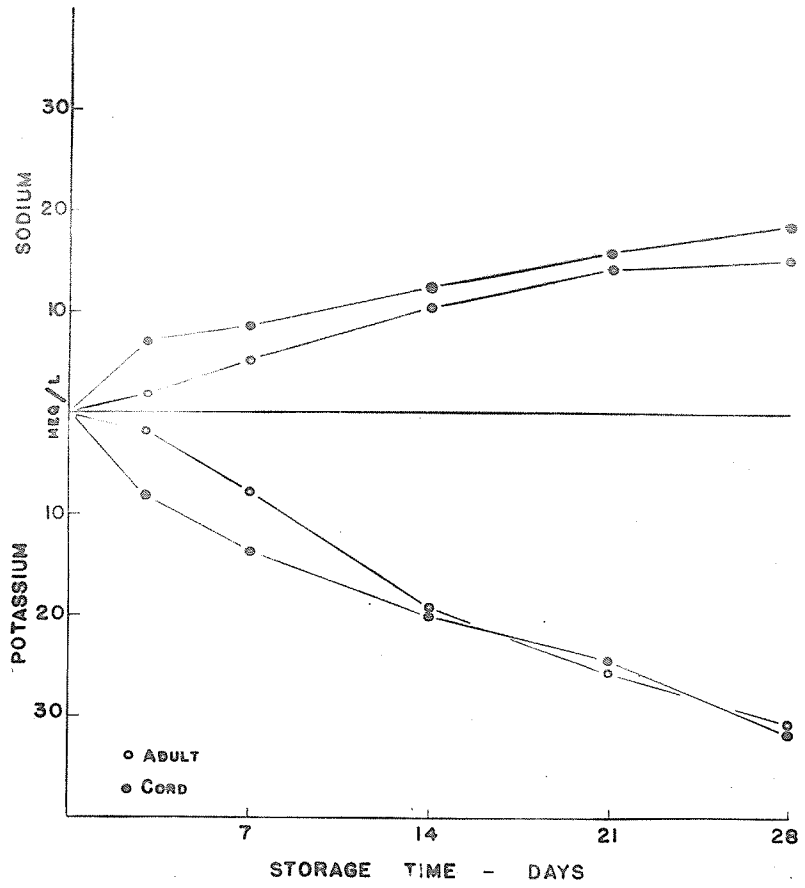
TABLE 2.

POTASSIUM CONCENTRATION IN STORED ERYTHROCYTES

(meq./l. of red cells)

Sample		Days of storage 0	3	7	14	21	28
Adult	1	85.3	81.6	75.1	63.2	59.4	54.7
	2	86.9	84.1	85.3	64.4	63.1	55.9
	3	83.3	82.5	77.6	64.2	58.2	58.9
	4	87.2	83.8	72.4	65.2	56.1	52.4
	5	83.6	80.0	76.5	73.4	61.9	51.9
Mean ± SD		85.3 ± 1.8	81.5 ± 2.0	77.4 ± 4.9	66.1 ± 4.2	59.8 ±2.8	54.8 ± 2.9
Cord	1	99.5	95.6	88.2	84.3	77.8	75.2
	2	85.8	78.6	80.1	69.5	64.7	49.2
	3	89.5	81.8	72.9	73.1	70.3	56.4
	4	90.8	83.8	76.5	64.0	60.0	61.0
	5	97.7	83.0	77.4	72.7	69.0	65.6
Mean ± SD		92.7 ± 5.7	84.6 ± 6.7	7.90 ± 5.8	72.7 ± 7.4	68.3 ± 6.6	61.5 ± 9.1

FIGURE III



CHANGES IN INTRACELLULAR ION CONCENTRATION

DURING STORAGE OF

ADULT AND CORD BLOOD

Each value is the mean of five samples

TABLE 3.

pH OF STORED BLOOD

Sample	Period of Storage (days)			
	0	7	14	28
Cord 1	7.01	6.72	6.64	6.62
2	7.13	6.67	6.70	6.56
3	7.10	6.73	6.72	6.60
Adult 1	7.13	-	6.65	6.61
2	7.15	-	6.61	6.59

TABLE 4.

INORGANIC PHOSPHATE CONCENTRATION OF STORED BLOOD

(mgms. P/100 ml.)

	Days of storage					
Sample	0	3	7	14	21	28
Adult 1	4.55	5.3	10.8	12.1	18.9	19.7
2	4.17	7.80	8.0	13.2	-	17.0
3	3.90	7.5	9.5	11.1	14.1	20.7
4	4.50	4.6	8.3	14.3	13.2	18.8
5	4.80	5.2	11.7	11.6	13.4	21.3
Mean ± SD	4.38 ± .31	6.08 ± 1.3	9.66 ± 4.1	12.5 ± 1.4	14.4	19.5 ± 1.6
Cord 1	-	-	11.5	13.5	15.2	18.0
2	3.32	8.1	10.7	12.6	17.1	19.6
3	4.07	8.5	12.2	12.4	15.1	-
4	4.17	7.76	13.7	15.7	18.1	20.5
5	3.84	6.0	12.4	16.0	15.4	17.1
Mean ± SD	3.85 ± .30	7.59 ± 1.1	12.1 ± 1.1	14.0 ± 1.8	16.2 ± 1.4	18.3 ± 1.7

TABLE 5.

PLASMA GLUCOSE CONCENTRATION
IN STORED BLOOD

Each value is expressed in mgms./100 mls. as the mean and standard deviation of three to five samples.

Period of storage (days)	Cord	Adult
0	471 ± 17	452 ± 18
3	435 ± 21	410 ± 25
7	373 ± 24	384 ± 31
14	319 ± 16	308 ± 11
21	282 ± 37	265 ± 21
28	260 ± 33	233 ± 26

TABLE 6.

ORTHOPHOSPHATE PARTITION IN CORD BLOOD

(mgms.P/100 ml.)

Sample	Term of Storage (days)	Whole Blood TASP	Plasma TASP	RBC TASP	Plasma IP	RBC IP	Plasma Organic Phosphate
1	0	23.5	5.6	44.0	4.6	-	1.0
	21	23.7	6.8	42.4	5.4	-	1.2
2	0	24.5	2.8	48.5	1.8	-	1.0
	21	23.1	7.5	43.0	6.2	-	1.3
3	0	20.0	2.9	42.9	2.1	-	0.8
	21	20.8	7.2	37.3	6.2	-	1.0
4	0	-	-	-	2.80	3.90	-
	14	-	-	-	5.75	15.4	-
	28	-	-	-	7.00	25.0	-
5	0	-	-	-	2.54	4.02	-
	7	-	-	-	5.80	10.9	-
	14	-	-	-	7.15	12.5	-
	28	-	-	-	8.85	23.5	-

TABLE 7.

ALKALI DENATURATION OF Hb F

Sample	Denaturation Rate (min^{-1}) Fresh	Stored	Alkali-Resistant Hb (%) Fresh	Stored
1	4.06	5.56	90	87
2	4.09	5.84	71	66
3	3.87	4.67	79	76
4	4.08	4.83	80	75
5	4.09	5.84	73	65
Mean	4.04 ± .09	5.35 ± .55		

$$\text{Denaturation Rate} = \frac{2.3}{\delta(\text{minutes})} \times \log_{10} \frac{\text{Hb concentration at 2 mts.}}{\text{Hb concentration at 10 mts.}}$$

from 11.8 ± 0.8 to 30.5 ± 1.6 in cord blood. In the first three days of storage the mean sodium gain in adult cells was 2.0 ± 1.2 and in cord cells was 6.7 ± 3.8. These values are significantly different (P .05) by "Students" t test (Snedecor, 1946).

Potassium concentrations are tabulated in Table 2. The intracellular potassium concentration was higher in cord blood: 92.7 ± 5.7 compared to 85.3 ± 1.8. These values fell in four weeks to 61.5 ± 9.1 and 54.8 ± 2.9 meq./l. red cells. The cord cells lost 8.3 ± 4.0 and the adult cells 2.8 ± 1.2 meq./l. in the first three days. These values are significantly different (P .05). The mean changes in sodium and potassium values are shown in Figure III.

Table 3 shows pH values of stored blood. The mean pH on the day of collection was 7.08 for cord blood and 7.14 for adult blood. The pH values dropped in four weeks to means of 6.59 and 6.60. Inorganic phosphate values are tabulated in Table 4. In adult blood the orthophosphate rose from 4.38 ± .31 to 19.5 ± 1.6 and in the cord blood from 3.85 ± .30 to 18.3 ± 1.7 mgms./100 ml. during four weeks storage. From the third to the twenty-first day of storage the mean level of inorganic phosphate was higher in cord blood but this is not statiscally

significant.

Table 5 shows the plasma glucose concentrations of stored cord and adult blood. The adult level fell from 452 ± 18 to 233 ± 26 mgms./100 mls.; in cord blood from 471 ± 17 to 260 ± 33 mgms./100 mls. There was no significant difference in glucose concentration but the mean value fell slightly more in adult blood during storage.

Orthophosphate values are tabulated in Table 6. In samples 1,2 and 3 the whole blood TASP concentrations did not change significantly during three weeks storage. In each case there is a decrease in intracellular TASP and a rise in plasma TASP.

In samples 4 and 5, the intracellular orthophosphate rose during four weeks storage from 3.9 to 25.0 and from 4.02 to 23.5 mgms./100 mls. There was smaller rise in plasma orthophosphate from 2.8 to 7.0 and from 2.54 to 8.85 mgms./100 mls.

Table 7 shows the changes during storage of the alkali denaturation rate of Hb F. In the five samples studied, the mean rate fell in three weeks from 4.04 ± .09 to 5.35 ± .55 min^{-1}. The concentration of alkali resistant hemoglobin fell from three to eight per cent.

Before discussing the results, we will investigate the possibility that Hb F has some effect on the ion metabolism of cord erythrocytes, since cord cells lost potassium more rapidly during the first three days of storage.

HEMOGLOBIN AND POTASSIUM DIFFUSION

Review: Over the years there has been considerable interest in the possible interactions of sodium and potassium with hemoglobin, and there has been some indication in the literature that the osmotic activity of these ions is decreased in the presence of hemoglobin (Peters, 1944). Stratmann and Wright (1946) claimed that equine hemoglobin retained potassium, and moreover could accumulate this ion against a concentration gradient.

Recently, however, experiment has indicated that there is no such binding. Morris and Wright (1954) found no evidence for the preferential binding of sodium or potassium, nor of alterations in their activities by the hemoglobins of human, horse or dog. Batlley and Klotz (1951) also showed by dialysis studies that neither ion was bound to horse hemoglobin, and Carr (1956) used a potentiometric technique to demonstrate that neither bovine nor human hemoglobin bound sodium or potassium. All these studies, however, were done

with hemoglobin concentrations very much lower than those found in the red cell.

Chromate ion is more readily eluted from Hb F (Suderman, White and Israels, 1957). Hb F has a greater affinity than Hb A for $Al(OH)_3$ (Betke, 1954). Otherwise no studies have been made on differences in ion binding power of Hb A and Hb F. To investigate the possibility that there is some ion binding with high hemoglobin concentration, and to search for a possible difference between Hb A and Hb F, a dialysis experiment was carried out using hemoglobin concentrations comparable to those found in the cell.

Method: Blood collected in ACD or heparin was centrifuged and the plasma and buffy coat removed. The cells were hemolysed by repeated freezing and thawing until a smear of the hemolysate examined microscopically showed no intact cells. Hemolysates were kept frozen until used.

Dialysis bags were made of Arthur H. Thomas brand cellulose, so constructed with Scotch brand masking tape that only two square inches of the membrane were exposed for diffusion (Figure IV.).

Five mls. of the hemolysate or of KCL solution were allowed to stand in the bag for fifteen minutes at

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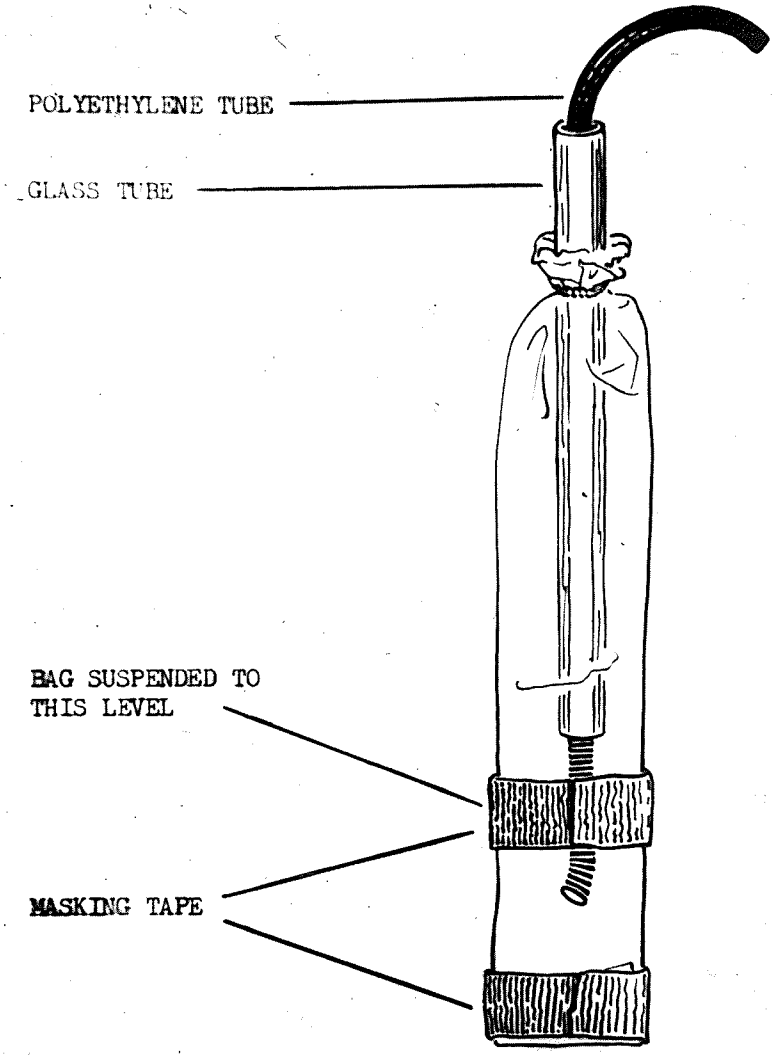
FIGURE IV



DIALYSIS BAG FOR MEASURING POTASSIUM DIFFUSION FROM HEMOLYSATES

room temperature. The bag was then suspended in one and a half liters of 0.9% (w/v) NaCL at $4^{\circ}C$ or $37^{\circ}C$. The saline was mechanically stirred and the contents of the bag stirred periodically by blowing into it through a polyethylene tube. Diffusion was allowed to proceed for thirty minutes.

Hemoglobin concentrations were determined colorimetrically and alkali-resistant hemoglobin (Hb F) was determined by the method of White, Delory and Israels (1950). Potassium concentrations were determined with the flame photometer on 0.1 ml. samples of hemolysate, removed from the bag at 10 or 15 minute intervals. It was found that diffusion was first order with respect to potassium concentration, and the rate constant was calculated from the equation:

$$\text{Rate} = \frac{2.3}{\text{time (minutes)}} \times \log_{10} \frac{\text{original potassium concentration}}{\text{potassium concentration}}$$

The rate given in the results for each sample is the mean of the two or three rates calculated from the two or three potassium determinations.

<u>Results:</u> From Table 8, it may be seen that Hb A and Hb F do not differ in any capacity they might have to bind potassium. The mean diffusion rates from the

TABLE 8.

POTASSIUM DIFFUSION RATES FROM KCL SOLUTIONS

AND HEMOLYSATES

Sample	Temperature °C	Hb Conc. gms. %	F Hb Conc. %	K Conc. meq./l.	Rate Constant 37°	Rate Constant 4°
KCL #1	37	-	-	100	.0538	
#2	37	-	-	100	.0537	
#3	37	-	-	100	.0530	
#4	37	-	-	78	.0496	
#5	37	-	-	77	.0544	
#6	4	-	-	100		.0295
#7	4	-	-	100		.0318
#8	37	-	-	10	.0521	
Mean					.0528	
S.D.					.0017	
Adult						
#1	37	28	-	91	.0229	
#2	37	31	-	92	.0283	
#3	37	25	-	78	.0186	
#4	37	29	-	91	.0214	
#5	37	28	-	77	.0219	
#6	4	30	-	99		.00730
#7	4	25	-	100		.00690
#8	37	27	-	79	.0231	
#9	37	23	-	77	.0225	
Mean					.0229	
S.D.					.0030	
Cord						
#1	37	21	77	85	.0366	
#1	37	21	77	85	.0340	
#2	37	31	78	80	.0253	
#3	37	22	63	99	.0169	
#4	37	29	62	110	.0207	
#5	37	27	59	91	.0180	
#6	4	34	55	99		.00778
#7	4	33	65	91		.00762
#8	37	29	73	94	.0171	
Mean					.0222	
S.D.					.0071	

Rate constants are expressed as minutes^{-1}.

TABLE 9.

POTASSIUM DIFFUSION RATES FROM HEMOLYSATES

Sample		Potassium Concentration (meq./l)	Diffusion Rate	In Vivo Exchange Rate
Bovine	1	18.5	.0250	3.8
	2	23.3	.0263	-
Rat	1	96	.0330	6.0
	2	101	.0288	-
Rabbit	1	114	.0275	3.0
Dog	1	7.0	.0231	1.0
Human Adult (Mean of 7)		83	.0229	1.8
Human Cord (Mean of 6)		93	.0222	-

Potassium diffusion rates are expressed as min^{-1}. The in vivo exchange rates (Sheppard, 1950) are expressed as % intracellular potassium/hour.

two series are almost identical: .0229 min.$^{-1}$ for adult blood; and .0222 min.$^{-1}$ for cord blood. Moreover, the potassium diffusion rate from hemolysates of bovine, canine, rat and rabbit erythrocytes do not differ in magnitude from those of the human (Table 9), despite the large differences in in vivo potassium exchange found by Sheppard (1950).

The potassium diffusion rate is not significantly effected by hemoglobin concentration ($r = +.64$) or by Hb F concentration ($r = +.67$).

It should be noted too that diffusion from hemolysates is markedly slower than from a KCL solution; the difference is highly significant ($P < .01$).

DISCUSSION

In the functioning erythrocyte in vivo, ion concentrations are kept constant since passive ion flow along the concentration gradient is equalled by the active ion transport of the cell. However, when the cell is cooled, as in storage, metabolism slows and passive diffusion predominates.

The more rapid change in intracellular ion concentration in cord cells may be due to three factors: a greater permeability of the membrane to passive ion flow, a lesion of the ion transport mechanism, or a more

rapid breakdown of the metabolic system supplying the energy for active ion transport.

There is no doubt that the cord red cell membrane suffers some damage during collection into ACD. By gross inspection, hemolysis was much more marked in cord blood than in adult blood, and it was for this reason that Sjolin did not use ACD as a preservative medium. It would be necessary to compare the rates of potassium efflux and sodium influx in cord samples collected into ACD and into heparin to determine if permeability is markedly changed during collection into ACD. If this were found to be so, it would substantiate Gibson's hypothesis that storage lesions orginate in damage suffered during collection (1956).

The second possibility, that there is a lesion of the ion transport system, could be investigated by measuring active cation transfer in samples collected into ACD and into heparin.

The third explanation of the more marked change in ion concentration in cord erythrocytes is that there is an earlier decrease in the source of energy. This would be hard to evaluate. Glycolysis continues in cord cells, and as will be shown later, there is ATP remaining

after three weeks storage.

The close proximity of the observed pH values of cord and adult blood indicate that pH is not a factor in the more marked ion lesion, of cord cells. Kashket, Rubinstein and Denstedt (1956) have suggested that the acid pH during ACD storage may be a contributing factor to the lesion of storage, since the enzymes hexokinase and glycerate-2,3-diphosphatase have an optimum pH of 7.8 and 7.0 respectively.

The diffusion experiment indicates that Hb F is not a contributing factor to potassium loss from the cord erythrocyte. The most interesting observation here is that potassium does not diffuse as rapidly from a hemolysate as from a salt solution. It may be that pores in the cellulose membrane are blocked by cell constituents, or that potassium is bound to some substance in the hemolysate that lowers its diffusion rate.

It should be noted that the inorganic phosphate concentrations on the day of collection were much higher than the "normal" values of two mgms./100ml., since in shed blood at room temperature phosphate esters breakdown to orthophosphate readily. We are, therefore,

not warranted in measuring changes in inorganic phosphate from the initial value. We cannot, therefore, confirm the observations of Rotfelt and Greenwalt and Ayers that inorganic phosphate is formed more rapidly in cord blood. However, we shall note in a later series of observations that the intracellular inorganic phosphate concentration is significantly higher in cord cells after storage.

The glucose values indicate that glucose metabolism is of the same order of magnitude in cord blood as in adult but the results do not allow us to determine if there is a significant difference in glucose utilization. The use of plasma glucose concentrations is possibly not valid as a criterion of red cell metabolism, and the whole blood glucose values would have been preferable.

The results of the orthophosphate diffusion experiment cannot be reconciled with the findings of Greenwalt and Ayers. There is no appreciable change in the TASP concentrations of whole blood during storage, indicating that the phospholipid fraction does not contribute to the acid soluble phosphate fraction. There is an increase of the TASP plasma and a corresponding decrease of intracellular TASP, the organic phosphate concentration in plasma remained constant within the error of the procedure.

It is apparent that in cord blood as in adult, storage results in a decrease in intracellular phosphate, due to an outward diffusion of orthophosphate along a concentration gradient.

The drop in alkali resistant hemoglobin may be due to the formation of methemoglobin F which is not alkaline resistant. However, there is no explanation for the increase in the rate of denaturation.

Our results indicate that in cord blood there is a defect in ion metabolism and other workers have reported differences in phosphate metabolism. Gabrio and her co-workers reported that adenosine reversed storage lesions in stored adult blood, replenishing phosphate esters and improving intracellular cation concentrations. It was therefore decided to investigate the effects of adenosine on the storage lesion of the cord erythrocyte.

PART D

ADENOSINE AND RED CELL METABOLISM

REVIEW

Adenosine and the storage lesion: In their experiments with adenosine, Gabrio and her co-workers incubated adenosine with blood after two or three weeks storage, and at two or three week intervals thereafter. In each case they found a repletion of intracellular organic phosphate during incubation and a higher concentration of organic phosphate during subsequent storage of adenosine treated samples. These workers used adenosine concentrations of 25×10^{-3} moles/liter of red cells, or approximately 10×10^{-3} moles/liter of whole blood.

Rubinstein, Kashket and Denstedt (1956) stored blood in ACD containing sufficient adenosine to give a final concentration of 0.4% (approximately 15×10^{-3} moles/liter of whole blood). They found that adenosine maintained a low inorganic phosphate concentration and a high level of 2,3-DPG, ATP and hexose phosphates through thirty-three days of storage. Prankerd (1956) also added adenosine on the day of collection (5×10^{-3} moles/liter of whole blood) and found that adenosine

maintained a higher organic phosphate level but did not have any effect on the ATP concentration. These contradictory results indicate that nucleoside concentration may have an appreciable effect on the nature of the phosphate partition.

Prankerd also found that adenosine did not change the glucose utilization of stored erythrocytes. This agrees with Gabrio's findings that the re-synthesis of organic phosphate with nucleosides arises mole for mole solely from the ribose moiety of adenosine.

Gabrio and her co-workers reported little on the effect of nucleosides on intracellular ion concentration. In one experiment, adenosine was added after twenty-one days storage, and it was noted that the adenosine treated sample had a higher intracellular potassium content after a further seven and twenty-one days. In another communication (Donahue, Finch and Gabrio, 1956), they reported that: "... determinations of the sodium-potassium gradient also indicated improved chemical characteristics of the erythrocyte when compared with control blood stored in ACD alone." Here the authors gave no numerical results.

Rubinstein et al (1956) did not measure ion concentrations in their experiments. Prankerd (1956)

reported that adenosine had no effect on intracellular sodium and potassium concentrations. Weisburg and Kaplan (1958) in a brief communication stated that inosine (1.8 gms./500 ml. of blood or approximately 15×10^{-3} moles/liter) caused plasma potassium values to rise more rapidly in samples stored with that nucleoside. On the other hand, Lange, Escobar and Crosby (1956) found that inosine did not effect plasma sodium or potassium.

Adenosine and cation transport: Kahn and Cohen (1957) studied the effects of nucleosides and related compounds on active potassium transport of erythrocytes incubated at 37°C after storage at 4°C. They found that adenosine and inosine augmented transport at concentrations lower than 5×10^{-3} moles/liter. Inosine reached a plateau of augmentation at this concentration while adenosine inhibited transport at higher concentrations. Adenine inhibited potassium transport at all concentrations while hypoxanthine and inosine-mono-phosphate were without effect. Extracellular ATP above 3×10^{-3} moles/liter also inhibited potassium transport.

Kahn and Cohen disregarded the possibility that adenosine *per se* inhibits potassium transport. They suggested that at concentrations over 5×10^{-3} moles/liter the deamination of adenosine by adenosine deaminase is rate-limited by the concentration of the enzyme. Then adenosine is transformed by some phosphorylase to ribose-phosphate and transport-inhibiting adenine. This is contrary to the findings of Gabrio *et al*, Rubinstein *et al* and Prankard who have found that adenosine is converted quantitatively to hypoxanthine via inosine. The reaction is much slower at $4^{\circ}C$ than at $37^{\circ}C$.

Substances which inhibit active ion transport across the cell membrane may be divided into three groups.

I. Enzyme inhibitors, such as sodium fluoride and iodoacetic acid. These substances block the glycolytic pathway, thereby depriving the cell of the high-energy phosphates necessary to maintain active ion transport.

II. Ion transport inhibitors, such as the cardiac glycosides. These substances block active sodium and potassium transport directly. They have no effect on glycolysis and are not affected by nucleosides.

III. Ion transport inhibitors which stimulate the

formation of organic phosphate. There is only one such compound reported. Jones (1958) found that deoxy-corticosterone glucoside increased the total phosphate and 2,3-DPG concentration of erythrocytes, while increasing potassium loss and sodium intake. He found no appreciable change in cell size.

The results obtained by Cohen and Kahn with adenosine place it in the same class with the adrenal hormone, although there is no apparent similarity between the two.

It was decided to observe the effects of adenosine on cord erythrocyte metabolism both during and after storage at 4°C by storing blood with adenosine, or incubating blood with adenosine after three weeks storage. This would determine whether:

1) Adenosine removes the differences previously noted between cord and adult blood during storage;
2) Adenosine has any effect on intracellular sodium and potassium concentrations.

THE REVERSIBILITY OF THE STORAGE LESION

Review: Since the erythrocyte membrane is only slightly permeable to the passive diffusion of inorganic

phosphate, there is an accumulation of this ion in the red cell during storage due to the breakdown of organic phosphate esters. However if cells are then incubated at 37°C, glucose utilization is enhanced and organic phosphate is formed, lowering the concentration of inorganic phosphate. If adenosine is added before incubation, the decrease in inorganic phosphate is more marked. Gabrio et al (1955) termed this the in vitro reversal of the storage lesion.

Greenwalt and Ayers (1956) found that cord erythrocytes after fourteen days storage could re-synthesize organic phosphate upon incubation. In blood storage, red cells are discarded after three weeks, when their survival in the recipient is 70%. Since this is the conventional "life" of stored blood, it was thought to be of interest to compare the abilities of cord and adult erythrocytes to form organic phosphates at this time and especially to note the effects of adenosine.

Method: Cord and adult blood was stored for twenty-one days in ACD at 4°C. After that time, samples were divided into three ml. fractions. To one was added 0.1 ml. of ACD. This fraction (A) was centrifuged and

TABLE 10

THE REPLETION OF ORGANIC PHOSPHATES DURING INCUBATION OF STORED BLOOD

IP	Inorganic Phosphate
EH	Easily Hydrolyzable phosphate
DH	Difficulty Hydrolyzable phosphate
NH	Non-Hydrolyzable phosphate
TASP	Total acid Soluble Phosphate

All concentrations are in mgms. P per 100 ml. packed red blood cells.

A	Blood stored 21 days
B	Blood stored 21 days, incubated 1 hour at 37°C
C	Blood stored 21 days, incubated with adenosine 1 hour at 37°C

TABLE 10A

ADULT BLOOD

Sample		IP	EA	DH	NH	TASP
1	A	19.8	4.2	2.4	8.0	34.4
	B	17.8	5.0	1.6	8.2	32.6
	C	8.6	9.8	6.6	11.1	36.1
2	A	20.0	5.6	1.9	3.7	31.2
	B	12.8	11.2	2.0	9.2	35.2
3	A	16.0	9.2	2.2	9.0	36.4
	B	13.6	6.4	1.4	13.3	34.7
4	A	17.4	9.2	1.8	6.2	34.6
	B	11.7	11.7	1.3	13.7	38.4
	C	7.6	11.8	7.3	8.1	34.8
5	A	16.0	5.6	2.1	13.8	37.5
	B	13.6	11.6	1.5	11.5	38.2
	C	3.2	9.8	6.3	17.0	36.3
6	A	21.0	3.1	2.5	6.6	33.2
	B	18.0	2.8	1.4	8.0	30.2
	C	6.3	5.9	4.8	16.8	32.8
7	A	19.0	.8	2.4	7.8	30.0
	B	16.0	.2	1.0	12.4	29.6
	C	4.8	3.4	5.2	16.2	29.6
8	A	19.2	1.6	1.4	5.8	28.0
	B	14.6	.4	1.4	12.0	28.4
	C	4.8	5.6	4.6	16.0	31.0
9	A	20.8	3.8	.8	7.2	32.2
	B	14.0	1.2	2.8	12.8	30.8
	C	4.1	6.5	5.4	15.6	31.6
10	A	20.8	1.6	1.0	12.6	36.0
	B	15.8	1.2	3.9	14.6	35.5
	C	5.6	5.6	8.0	16.8	36.0

TABLE 10B

CORD BLOOD

Sample		IP	EH	DH	NH	TASP
1	A	20.0	4.0	2.6	11.6	38.2
	B	15.6	6.0	0.1	13.1	34.6
	C	6.6	7.2	6.2	16.2	36.2
2	A	21.0	2.4	1.8	11.2	36.4
	B	18.2	4.8	3.2	6.4	32.6
	C	8.0	8.0	5.4	19.0	40.4
3	A	21.4	3.0	1.0	9.9	34.8
	B	17.6	5.2	1.8	10.2	34.8
	C	9.6	3.2	6.6	16.6	36.0
4	A	19.8	3.2	1.8	5.2	31.0
	B	16.0	6.8	5.4	5.8	34.0
	C	6.6	8.0	4.2	17.8	36.6
5	A	20.6	3.4	1.2	10.6	35.8
	B	21.2	3.2	1.0	10.6	36.0
	C	7.7	5.5	5.8	17.4	36.4
6	A	22.8	3.0	0.8	8.8	35.4
	B	22.4	0.4	1.2	11.8	35.8
	C	7.4	4.8	7.0	16.8	36.0
7	A	25.4	1.2	0.6	4.8	32.0
	B	22.4	1.0	1.8	7.0	32.2
	C	7.7	5.5	6.0	15.2	36.4
8	A	23.4	1.8	0.2	7.8	33.2
	B	19.2	0.8	1.2	9.2	30.4
	C	5.3	3.5	2.0	12.2	37.0
9	A	20.8	2.0	1.6	7.0	31.4
	B	19.2	1.2	0.4	12.0	32.8
	C	6.0	4.4	6.2	11.8	28.4
10	A	19.2	1.4	4.6	6.8	32.0
	B	14.8	2.6	0.4	11.2	28.0
	C	5.8	4.8	5.4	13.2	29.2
11	A	21.0	1.0	0.8	9.8	32.6
	B	17.4	3.4	2.0	13.2	36.0
	C	4.7	5.7	2.8	19.8	33.0
12	A	25.8	0.8	0.6	4.8	32.0
	B	13.4	3.0	1.8	11.0	29.2
	C	4.5	4.1	3.4	20.8	32.8

TABLE 10C

		No. of Samples	IP	EH	DH	NH	TASP
	Adult Blood						
A	Stored 21 days	10	19.0 ± 1.9	4.4 ± 3.0	1.8 ± .6	8.1 ± 3.1	33.4 ± 3.1
B	Incubated 37°C	10	14.8 ± 2.1	5.2 ± 4.5	1.8 ± .9	11.6 ± 2.3	33.4 ± 3.6
C	Incubated + adenosine	8	5.6 ± 1.8	7.3 ± 2.8	6.0 ± 1.2	14.7 ± 3.3	33.5 ± 2.6
	Cord Blood						
A	Stored 21 days	12	21.7 ± 2.1	2.3 ± 1.1	1.5 ± 1.2	8.1 ± 2.5	33.7 ± 2.3
B	Incubated 37°C	12	18.1 ± 2.9	3.2 ± 2.0	1.6 ± 1.4	10.1 ± 2.4	33.0 ± 2.7
C	Incubated + adenosine	12	4.4 ± 3.0	5.4 ± 1.6	5.1 ± 1.7	16.4 ± 2.9	34.9 ± 3.4

a portion of packed cells prepared for phosphate analysis.

To two other fractions were added 0.1 ml. of ACD (B) or 0.1 ml. of ACD containing 2.5×10^{-5} moles of adenosine (C). These fractions were incubated for one hour at 37°C, then portions of packed cells taken for phosphate analysis. Samples were analysed for the following fractions:

1) Inorganic phosphate
2) Easily hydrolyzable phosphate - mainly ATP and ADP
3) Difficulty hydrolyzable phosphate - mainly AMP and hexose phosphate
4) Non-hydrolyzable phosphates - mainly 2,3-diphosphoglyceric acid

Results: The data for adult and cord samples are given in Tables 10A and 10B, and the means and standard deviations are compiled in 10C.

After three weeks storage at 4°C, the mean inorganic phosphate concentration of cord erythrocytes is 21.7 ± 2.1 mgms./100 mls., while that of adult cells is 19.0 ± 1.9. The easily hydrolyzable fraction is 2.3 ± 1.1 in cord cells and 4.4 ± 3.0 in adult cells. In each case, the difference between adult and cord blood is statistically significant ($P < .01$). There is no statistical difference

between adult and cord blood in the other fractions.

During incubation, the inorganic phosphate values fall 4.21 ± 1.9 and 3.65 ± 3.2 mgms./100 ml. in adult and cord cells. The addition of adenosine results in falls of 13.6 ± 2.2 and 14.9 ± 2.6 mgms./100 ml. in adult and cord cells. There is no significant difference in the inorganic phosphate changes of cord and adult blood.

THE STORAGE OF CORD BLOOD WITH ADENOSINE

Method:

Series I: Samples of blood were collected in ACD and transferred in 3.5 ml. fractions to sterile evacuated tubes. Control tubes contained no additive while adenosine tubes contained sufficient nucleoside in 0.1 ml. of water to give a final concentration of 12.5×10^{-3} moles/liter of whole blood. Samples were stored immediately at $4^{\circ}C$. Intracellular sodium and potassium, hematocrits, inorganic and non-hydrolyzable phosphate fractions and pH were measured on the day of collection and after seven, fourteen and twenty-eight days of storage. Glucose concentrations were measured on the day of collection and after twenty-eight days storage.

TABLE 11

pH OF CORD BLOOD STORED WITH ADENOSINE

Series I (12 x 10^{-3} moles adenosine)

Time of Storage (days)	pH	
	Control	Adenosine
0	7.04 ± .06	
7	6.69 ± .04	6.73 ± .19
14	6.67 ± .18	6.71 ± .14
28	6.61 ± .04	6.67 ± .14

Values are mean and standard deviation of five samples.

TABLE 12.

GLUCOSE CONCENTRATION IN CORD BLOOD STORED WITH ADENOSINE

Series I.

Period of Storage (days)	Glucose Concentrations
0	448 ± 33
28	215 ± 46
28 + adenosine	218 ± 48

Values are expressed in mgms./100 mls. whole blood, and represent the mean and standard deviations of five samples.

TABLE 13A

HEMATOCRITS OF STORED BLOOD

Series I (12.5×10^{-3}M adenosine)

	Period of Storage (days)			
Control	0	7	14	28
Cord 1	45.5	44.5	44.5	44
2	60.0	56.5	58.0	54.5
3	55.5	54.0	49.0	50.5
4	45.5	43.5	52.0	46.5
5	42.0	47.6	33.0	43.0
Adult 1	40.5	40.0	39.5	39.5
Adenosine				
Cord 1	-	38.0	39.5	39.5
2	-	50.5	46.0	49.0
3	-	43.5	44.5	39.0
4	-	42.0	38.0	32.5
5	-	32.0	25.5	25.5
Adult 1	-	35.5	34.5	34.0

TABLE 13B

HEMATOCRITS OF STORED BLOOD

Series II (5×10^{-3}M adenosine)

		Period of Storage (days)			
Control		0	7	14	28
Cord	1	45	45	44.5	44.0
	2	45	45	44.5	44.5
	3	50	49.5	49.5	47.0
Adult	1	39.5	-	39.5	39.0
	2	43.0	-	42.5	42.0
Adenosine					
Cord	1	-	44.0	43.0	42.0
	2	-	43.5	42.0	41.0
	3	-	48.5	47.5	45.5
Adult	1	-	-	36.5	34.5
	2	-	-	39.5	37.0

TABLE 13C

HEMATOCRITS OF STORED BLOOD

	Period of Storage (days)			
Series I	0	7	14	28
5 Cord	49.5 ± 7.6	47.6 ± 7.3	46.7 ± 9.2	47.3 ± 4.6
5 Cord + adenosine	-	41.3 ± 7.1	37.2 ± 8.5	34.1 ± 10.0
1 Adult	40.5	40.0	39.5	39.5
1 Adult + adenosine	-	35.5	34.5	34.0
Series II				
3 Cord	46.5 ± 2.7	46.5 ± 2.6	46 ± 2.9	45 ± 2.1
3 Cord + adenosine	-	46.0 ± 3.0	45 ± 3.1	43 ± 2.6
2 Adult	41.0	-	40.0	38.5
2 Adult + adenosine	-	-	38.0	35.5

TABLE 14.

HEMOGLOBIN CONCENTRATION OF CORD PLASMA

(gms. Hb/100 mls. plasma)

Series I

Sample		Period of Storage (days)		
		0	14 (Control)	14 (Adenosine)
Cord	1	.17	.25	.18
	2	.07	.19	.15
	3	.08	.42	.21
	4	.09	.13	.23
	5	-	.30	.33
Mean		.10±.03	.26±.11	.22±.07

TABLE 15A

SODIUM CONCENTRATIONS OF STORED RED CELLS

(meq./l. red blood cells)

Series I

		Period of Storage (days)			
Control		0	7	14	28
Cord	1	13	19	23	24
	2	12	17	29	37
	3	13	16	29	30
	4	14	20	27	34
	5	11	14	25	30
Adult	1	15	18	25	33
Adenosine					
Cord	1	-	35	37	45
	2	-	37	36	47
	3	-	33	38	41
	4	-	39	47	47
	5	-	26	30	41
Adult	1	-	31	34	45

TABLE 15B

SODIUM CONCENTRATIONS OF STORED RED CELLS

(meq./l. red blood cells)

Series II

		Period of Storage (days)			
Control		0	7	14	28
Cord	1	11.5	17.0	23.8	27.5
	2	10.5	15.6	26.3	25.3
	3	11.0	15.2	21.7	22.1
Adult	1	12.1	-	20.4	28.0
	2	10.3	-	18.0	34.0
Adenosine					
Cord	1	-	24.2	32.3	34.3
	2	-	27.2	35.1	26.6
	3	-	32.1	33.1	40.8
Adult	1	-	-	21.1	29.8
	2	-	-	24.0	40.8

TABLE 15C

SODIUM CONCENTRATION OF STORED RED CELLS

(meq./l. red blood cells)

No. of Samples	Period of Storage (days) 0	7	14	28
Series I				
5 Cord	13 ± 0.4	17 ± 2.3	27 ± 2.7	31 ± 5.1
5 Cord + 12.5M adenosine	-	30 ± 5.8	37 ± 6.1	44 ± 2.7
1 Adult	15	18	25	33
1 Adult + 12.5M adenosine	-	31	34	45
Series II				
3 Cord	11.0 ± 0.7	15.9 ± 0.4	23.9 ± 2.5	33.7 ± 2.1
3 Cord + 5M adenosine	-	27.8 ± 5.0	33.5 ± 4.7	34.2 ± 11
2 Adult	11.2	-	19.2	31.5
2 Adult + 5M adenosine	-	-	22.6	35.3

TABLE 16A

POTASSIUM CONCENTRATION OF STORED RED CELLS

(meq./l. red blood cells)

Series I (12.5M adenosine)

		Period of Storage (days)			
Control		0	7	14	28
Cord	1	102	79	83	64
	2	90	81	72	56
	3	94	90	78	56
	4	96	80	73	63
	5	107	77	76	56
Adult	1	85.0	81.8	63.9	56.8
Adenosine					
Cord	1	-	64	59	55
	2	-	63	52	44
	3	-	78	62	48
	4	-	60	54	47
	5	-	70	56	48
Adult	1	-	67	64.2	48.0

TABLE 16B

POTASSIUM CONCENTRATION OF STORED RED CELLS

(meq./l. red blood cells)

Series II (5×10^{-3}M adenosine)

		Period of Storage (days)			
Control		0	7	14	28
Cord	1	84	75.0	64.2	62.5
	2	94.2	73.6	75.8	70.5
	3	82.4	76.9	69.1	60.2
Adult	1	80.4	-	63.9	56.8
	2	85.7	-	72.8	59.0
Adenosine					
Cord	1	-	68.8	52.0	48.5
	2	-	54.3	51.0	46.6
	3	-	58.1	55.4	48.4
Adult	1	-	-	64.4	54.9
	2	-	-	65.0	55.5

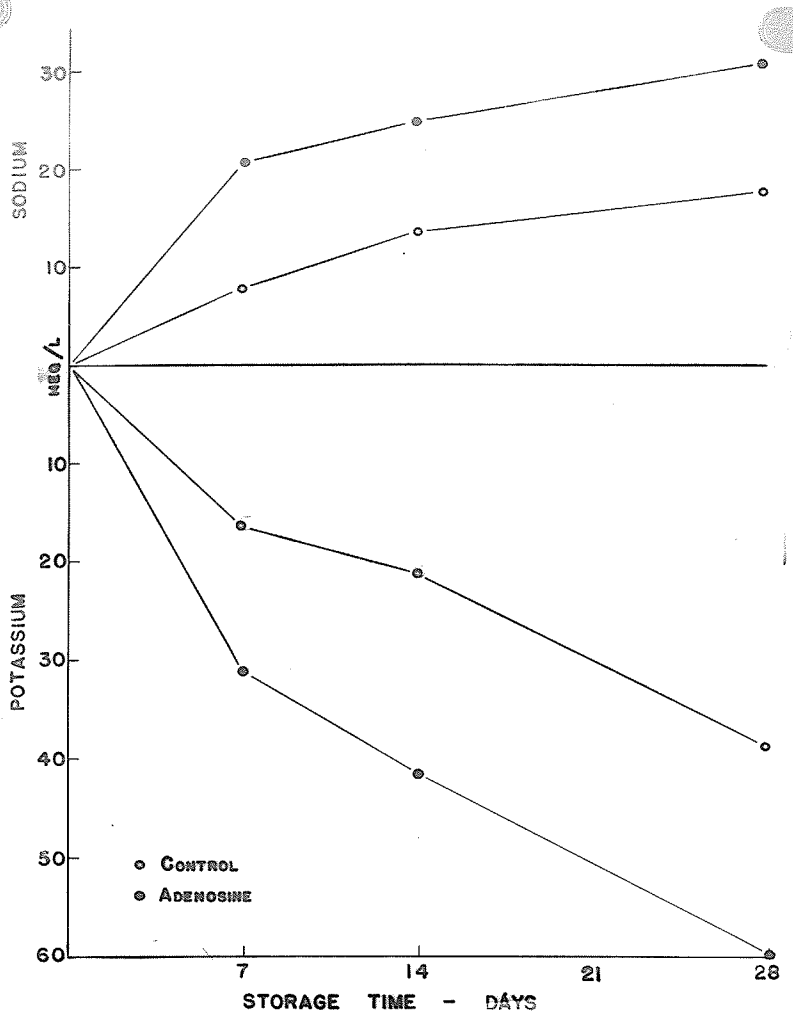
TABLE 16C

POTASSIUM CONCENTRATION OF STORED RED CELLS

(meq./l. red blood cells)

Samples	Period of Storage (days) 0	7	14	28
Series I				
5 Cord	97.9 ± 6.7	81 ± 5.0	76 ± 4.4	59 ± 4.1
5 Cord + 12.5M adenosine	-	67 ± 6.5	57 ± 4.0	49 ± 4.1
1 Adult	85.0	81.8	63.9	56.8
1 Adult + 12.5 M adenosine	-	67	64.2	48.0
Series II				
3 Cord	86.7 ± 5.6	75.2 ± 1.7	67.7 ± 6.11	64.4 ± 5.3
3 Cord + 5 M adenosine	-	60.4 ± 1.3	52.8 ± 3.6	47.8 ± 2.6
2 Adult	83.1	68.4	57.9	
2 Adult + 5 M adenosine	-	-	64.7	55.2

FIGURE V



CHANGES IN INTRACELLULAR ION CONCENTRATION

DURING STORAGE OF

CORD BLOOD WITH ADDED ADENOSINE

Each value is the mean of five samples

TABLE 17 A

PHOSPHATE PARTITION IN STORED BLOOD

NON-HYDROLYZABLE PHOSPHATE (SERIES I)

(mgms.P/ml. blood)

No. of Samples	Period of Storage (days) 0	7	14	28
Series I				
7 Cord	15.30 ± 1.41	7.50 ± 2.64	5.43 ± 3.48	1.64 ± 4.39
7 Cord + 12.5M adenosine	-	13.8 ± 3.41	12.4 ± 2.42	11.7 ± 4.81
1 Adult	12.70	9.10	5.20	0.35
1 Adult + 12.5M adenosine	-	12.65	10.10	9.35

TABLE 17B

INORGANIC PHOSPHATE IN STORED BLOOD

(mgms.P/100 ml.)

	Period of Storage (days)			
Control	0	7	14	28
7 Cord	3.26 ± .44	9.53 ± 1.83	14.07 ± 1.80	19.65 ± 1.40
1 Adult	3.2	6.60	10.80	17.60
3 Cord	2.07 ± .41	9.80 ± 1.0	15.8 ± 1.1	20.0 ± 1.4
2 Adult	3.05	-	8.65	16.20
Adenosine				
7 Cord + 12mM adenosine	-	3.99 ± 1.64	4.14 ± 1.60	5.79 ± 1.21
1 Adult + 12mM adenosine	-	3.90	4.50	8.85
3 Cord + 5mM adenosine	-	3.33 ± 3.9	10.9 ± 1.5	16.3 ± 2.1
2 Adult + 5mM adenosine	-	-	8.65	16.20

TABLE 18.

INORGANIC PHOSPHATE CONCENTRATION

Series III

		Adenosine	
No. of Samples	Control	5mM/liter	125 mM/liter
4 adult	14.1 ± 1.5	7.4 ± 1.6	3.0 ± .4
7 cord	14.6 ± 2.0	11.8 ± 1.1	3.9 ± .4

Phosphate concentration in mgms./100 mls. whole blood after fourteen days storage.

Series II: Samples of blood were collected in ACD and transferred in 3.5 ml. fractions to evacuated tubes. Control tubes contained 0.1 ml. of ACD. Sample tubes contained sufficient adenosine in ACD to give a final concentration of 5×10^{-3} moles/liter of whole blood. Samples were stored immediately. Hematocrits, inorganic phosphate, and intracellular sodium and potassium concentration were determined on the day of collection and after fourteen and twenty-eight days of storage at $4^{\circ}C$.

Series III: Control tubes contained 0.2 ml. of ACD. Sample tubes contained sufficient adenosine in 0.2 ml. of ACD to give a final concentration of 5×10^{-3} or 12.5×10^{-3} moles/liter of whole blood. Samples were stored immediately at $4^{\circ}C$. Inorganic phosphate levels were measured after fourteen days storage.

Results: pH values for the blood samples of Series I are shown in Table 11. The mean pH of five cord samples collected into ACD was $7.04 \pm .06$, and fell to $6.61 \pm .04$

after four weeks storage. The addition of 12.5×10^{-3}M adenosine significantly ($P < .01$), raised the pH during storage, and the mean value of adenosine treated samples after four weeks storage was 6.67 ± .14.

Glucose concentrations of the blood are shown in Table 12. On the day of collection, the control samples had a mean concentration of 448 ± 33 mgms./100 ml. This value fell during four weeks storage to 215 ± 46 mgms./100 ml. The storage of blood with adenosine did not significantly effect glucose metabolism, and the concentration was 218 ± 48 mgms./100 ml.

The hematocrits of five cord and one adult sample in Series I are tabulated in Table 13A while those of three cord and two adult samples of Series II are tabulated in Table 13B. The means and standard deviations are compiled in Table 13C.

In Series I, the mean hematocrit fell from 49.5 ± 7.6 to 47.3 ± 4.6 in four weeks storage; during this time the adenosine - treated samples fell to 34.1 ± 10.0. In Series II, the shrinkage is not so marked; the hematocrit fell from 46.5 ± 2.7 to 45.0 ± 2.1 in four weeks and adenosine - treated samples shrank to 43.0 ± 2.6.

Plasma hemoglobin concentrations are listed in Table 14. The mean hemoglobin concentration in plasma on the day of collection was .10 ± .03 gms./100 mls. After two weeks storage it was .26±.11 in the control samples and .22 ± .07 in the adenosine samples.

The sodium and potassium concentrations of red cells during storage in Series I and Series II are tabulated in Tables 15 A,B,C and 16 A,B and C. The intracellular sodium concentrations rose during storage from 13 ± .4 meq./l. to 31 ± 5.1 in Series I and from 11.0 ± .7 to 33.7 ± 2.1 in Series II of the cord samples. In the adenosine treated samples, the sodium concentration was much higher after four weeks storage - 44 ± 2.7 and 34.2 ± 11 in the two series. The sodium concentrations were significantly different in the adenosine treated samples ($P < .01$). It should also be noted that the sodium content was much higher in Series I adenosine sample than in Series II.

In Table 16C, it may be seen that adenosine caused the potassium concentration to become significantly lower ($P < .01$). In Series 1, the concentration fell to 59 ± 4.1 in the controls and to 49 ± 4.1 in the adenosine

samples after four weeks storage from an initial concentration of 97.9 ± 6.7 meq./l.

The mean change in sodium and potassium values of five Series I samples is shown in Figure V. After four weeks storage the mean sodium gain was 18 and 31 meq./l., and the mean potassium loss was 38.6 and 59.3 meq./l. in the controls and adenosine samples respectively.

Table 17 A shows the 2,3-DPG concentration in the samples of Series I. The concentration fell from 15.30 ± 1.41 to 1.64 ± 4.39 during four weeks storage while adenosine maintained a high level of 11.7 ± 4.81 mgms./100 ml. The 2,3-DPG concentration in the adult sample fell from 12.70 to 0.35, while adenosine produced a concentration of 9.35 after four weeks.

Inorganic phosphate concentrations are shown in Table 17B. In Series I, with the higher adenosine concentration, the orthophosphate concentration rose from 3.26 ± .44 to 19.65 ± 1.40 in seven cord samples and from 3.2 to 17.60 in an adult sample during four weeks storage. In samples stored with adenosine, the values only rose to 5.79 ± 1.21 and 8.85 mgms./100 mls. In Series II, the effect of adenosine was less marked.

In three cord samples, inorganic phosphate rose from 2.07 ± .41 to 20.0 ± 1.4 in controls and to 16.3 ± 2.1 in adenosine samples after four weeks storage.

After fourteen days storage with 12.5×10^{-3} M adenosine, the inorganic phosphate values in cord and adult blood were 3.9 ± .4 and 3.0 ± .4 mgms./100 ml. after two weeks storage. With only 5×10^{-3}M adenosine, the values were 11.8 ± 1.1 and 7.4 ± 1.6 mgms.100 ml.

NUCLEOSIDES AND RED CELL VOLUME

The lowering of hematocrit values by adenosine, noted in the preceeding section, has never been reported. Before discussing possible causes of this phenomenon, it was decided to determine if this property was shared by other nucleosides, and whether it arose from impurities in the adenosine or from breakdown products formed from adenosine during autoclaving.

Method: Adenosine, inosine, and guanosine (Nutritional Biochemicals Corporation) were prepared in the concentration 1 mg./1ml. of double distilled water. A portion of these solutions was autoclaved at 115°C for thirty minutes. The solutions were diluted 250-fold in pH 7 buffer (Beckmann #3581) and the absorption spectra

determined over the range 210-300mu using a Beckman DK-1 Recording Spectrophotometer. The position of the maxima and minima were compared to the published values of Beaven, Holiday and Johnson (1955).

400 ml. of adult blood were collected into 100 ml. of ACD, and 10 ml. aliquots were transferred to sterile evacuated tubes. Control tubes contained .25 ml. of ACD, and samples tubes contained sufficient nucleoside suspended in .25 ml. of ACD to give a final nucleoside concentration of 5 or 12.5 + 10^{-3} moles/liter of whole blood. A sample of blood was also collected in heparin. Samples were stored immediately at $4^{o}C$.

Mean corpuscular volumes were determined on a control and the heparinized samples on the day of collection. Hematocrits were determined on controls and samples after fourteen and twenty-eight days storage at $4^{o}C$. Since there was no hemolysis, the red cell count was assumed to be that of the initial control samples, and the MCV's were determined.

<u>Results:</u> The positions of the maxima and minima for ultra-violet absorption are tabulated in Table 19. The proximity of the values to the published values indicate that the nucleosides were pure. Neither the

TABLE 19

POSITION OF ABSORPTION MAXIMA AND MINIMA OF NUCLEOSIDES

	Published Value		Determined		Determined after Autoclaving	
	Δ Max	Δ Min	Δ Max	Δ Min	Δ Max	Δ Min
Adenosine	259	227	259	227	259	227
Adenine	262	224	-	-	-	-
Guanosine	253	224	252	223	252	223
Inosine	248	224	249	224	249	224
Guanine	246,276	224,263	-	-	-	-
Hypoxanthine	239	223	-	-	-	-
Xanthine	266	239	-	-	-	-

TABLE 20.

MCV's OF RED CELLS STORED WITH NUCLEOSIDES

(MCV EXPRESSED IN CUBIC MICRONS)

Sample	Day 0		Day 14							Day 28						
	Heparin	ACD	ACD	Adenosine		Guanosine		Inosine		ACD	Adenosine		Guanosine		Inosine	
				5	12.5	5	12.5	5	12.5		5	12.5	5	12.5	5	12.5
1	84	112	104	98	90	95	99	101	98	101	98	90	99	99	99	98
2	92	109	106	100	92	105	103	105	100	103	98	91	101	101	100	101

optical densities nor the positions of maxima and minima were changed by autoclaving.

Table 20 shows the MCV's of two samples of adult blood stored with nucleosides. With 5×10^{-3}M adenosine, shrinkage was from three to six cubic microns; with 12.5×10^{-3}M adenosine - eleven to fourteen cubic microns more than the control values on the corresponding day. With the exception of 5×10^{-3}M guanosine on day 14 in sample #1, inosine and guanosine did not lower the hematocrit as much as adenosine of corresponding concentration.

DISCUSSION

The reversibility of the storage lesion: It may be noted in Tables 10A and 10B that there was a wide variation between samples in the effect of incubation. Prankerd (1955) has pointed out this variability, but can offer no explanation for it. These deviations were probably increased by the difficulties in obtaining an accurate aliquot of packed red cells.

The cord samples had a statistically higher concentration of orthophosphate and a lower concentration of easily hydrolyzable phosphates than adult red cells. This agrees with the work of Greenwalt and Ayers (1956) who claimed that non-hydrolyzable phosphate fractions

fell more rapidly in cord blood, and inorganic phosphate rose more rapidly during three weeks storage in ACD at $4^{o}C$.

Cord and adult blood did not differ in their organic phosphate synthesis during incubation. This indicates that the ability of cord cells to synthesize organic phosphates is not less than that of adults. In this case, the more rapid fall-off of phosphate esters during storage would not be due to a decreased production but an increased utilization of high energy phosphates in cord blood.

Adenosine and phosphate metabolism: Both Prankerd (1956) and Rubinstein et al (1956) claimed that adenosine had no significant effect on glucose consumption in stored blood. It has been suggested that glycolysis slows in stored blood due to a decrease of ATP available for the phosphorylation of glucose. Since adenosine, in sufficient quantities, increases ATP concentration, it should result in an increase in glucose consumption. This apparently is not the case.

The phosphate values obtained in Series I, with 12.5×10^{-3}M adenosine, indicate that at this concentration

the nucleoside maintains a high 2,3 DPG and low orthophosphate concentration comparable to adult blood. The inorganic phosphate concentrations of adenosine treated samples in Series II were higher than in Series I. In Series III, although a high nucleoside concentration abolishes differences between cord and adult blood, the lower concentration is not as effective in cord blood in reversing the lesion in phosphate metabolism.

We have already noted that Rubinstein and Prankerd used different nucleoside concentrations and obtained different phosphate partition results. The results obtained here further indicate that nucleoside concentration is an important factor in reversing the lesion of storage. This is especially true in cord blood where, apparently, a low adenosine concentration is not as effective as in adult blood.

Adenosine and ion metabolism: The plasma hemoglobin concentration in adenosine treated samples was slightly less than that of the controls. This was probably due to a dilution of the pigment due to the decreased hematocrit. In any case, it indicates that the lowering of the hematocrit is not due to hemolysis

but to a shrinkage of red blood cells.

A decrease in erythrocyte size must be due to the loss of water. Since the membrane is freely permeable to water, the cell shrinkage we have noted must be due to a decrease in the number of ions in the cell, resulting in an efflux of water to maintain osmotic pressure.

Our ultra-violet absorbtion studies indicate that the cell shrinkage and altered ion metabolism were not caused by impurities, but stem from adenosine. Since Gabrio et al, Rubinstein et al and Prankerd have all found that adenosine is converted to inosine and hypoxanthine, and not to adenine, it appears from studying our results and those of Kahn and Cohen that adenosine per se, or its adenine moiety, inhibits active potassium transport. Since the cell membrane is less permeable to sodium than to potassium, there is a decrease in total intracellular cation, and hence a decrease in cell size.

Harris and Maizels, (1952) have pointed out that a human erythrocyte lacking an active potassium transport system would shrink and become so acid inside that the ability of hemoglobin to transport respiratory gases would be impaired. No attempt was made to determine the intracellular pH of the adenosine treated samples.

It is necessary to determine why our results differ from those of Gabrio et al and Prankerd. Gabrio incubated the blood and adenosine for one hour at 37°C.; Prankerd does not state if he incubated his samples prior to storage, but it would appear from his cation results that he did. In these cases, the adenosine would be rapidly converted to the innocuous inosine. However, in our experiment, without prior incubation, the adenosine is only slowly converted to inosine at 4°C, and therefore, especially at high concentrations, may readily inhibit the active potassium transport. pH values indicate that at least some adenosine is deaminated.

The suggestion that the adenosine per se or its adenine moiety is the inhibiting agent is supported by Kahn and Cohen's observation that extracellular ATP, which is not de-aminated, can also inhibit transport.

We cannot tell if the slight RBC shrinkage in two samples with inosine and guanosine is due to an osmotic effect or an inhibition of ion metabolism. If these nucleosides have a marked effect on potassium transport, they would then have some disadvantages as metabolic adjuvants during storage. While they may stimulate organic phosphate transport and prolong red

cell viability, the high plasma potassium concentration would be dangerous in a massive transfusion. Gabrio and her co-workers found that adenosine stimulated phosphate ester formation equally well if it was not incubated. However, our results show that incubation might be very important in the use of nucleosides as red cell nutrients during blood preservation.

PART E

CONCLUSION

DISCUSSION OF RESULTS

It might be well to discuss our results in light of our original aim and scope of the project. This was two-fold: to compare the storage lesion in adult and cord blood, and to find if Hb F had any effect on cell metabolism.

Our results seem to indicate that the lesions of storage in cord and adult blood do not differ in nature but only in degree, especially during the first few days of storage. It may be that the cord erythrocyte is depleted more rapidly of some metabolite, or that it is more susceptible to the "lesion of collection". Our incubation experiment indicates that the fault does not lie in a reduced ability in cord cells to synthesize phosphate esters.

The statement that the lesions do not differ in nature is borne out by the fact that adenosine, at sufficient concentration, corrects the lesion in phosphate metabolism equally well in cord and adult blood.

The observation that Hb F loses its alkali resistance during storage cannot be explained by present

knowledge. It is interesting to speculate that hemoglobin structure may be maintained by erythrocyte metabolism. However this lies outside the scope of the thesis, and is probably not important in the problem of maintaining red cell viability during storage.

We have gained no positive knowledge about the possible effects of hemoglobin on red cell metabolism. Our results indicate that it has no effect on potassium diffusion. Further experiments were planned to test the effect of hemoglobin on blood storage, but these were abandoned in favour of work on nucleosides and cell cation concentrations.

This latter study was not envisaged in our original aim, but it was carried out for two reasons. The first is that the literature contains contradictory results of the action of nucleosides on cation concentration. Our results indicate that the ion transport may be affected both by adenosine concentration and by the treatment of the blood sample before storage.

Secondly, sodium and potassium transport and exchange is important in many branches of physiology, and the red cell is a popular medium for testing the effects of transport inhibitors, such as the cardiac glycosides.

The discovery that with adenosine we may "divorce" glycolysis and ion transport is very interesting. It is generally stated that during storage active cation transport is slowed because of a decrease in avaiable high energy phosphates. However with adenosine, the phosphate esters are kept at a high concentration while cation lesions are either not effected (Prankerd) or worsened. This contradictory effect of adenosine on phosphate and cation metabolism during storage deserves further study.

The fact that adenosine may block active ion transport may be of some practical value in studying cation fluxes in erythrocytes. Whereas some transport inhibitors poison glycolysis, or have no effect on it, adenosine might be useful experimentally to block active ion transport while maintaining phosphate metabolism at normal levels for prolonged periods of time.

Our results indicate that both phosphate metabolism and cation transport are sensitive to nucleoside concentration, and to incubation prior to storage. This may explain the contradictory results noted in the literature.

SUMMARY OF RESULTS

1. The lesions of storage were studied in cord and adult blood samples stored at 4°C in ACD. During the first few days of storage the cord erythrocytes lose more potassium and gain more sodium than adult red cells. After this period there is no significant difference in ion concentration changes.

2. During storage there is a decrease in intracellular TASP in cord erythrocytes due to an outward diffusion of orthophosphate.

3. There is a decrease in the characteristic alkali resistance of Hb F in cord blood during storage.

4. The nature of the hemoglobin has no effect on the diffusion of potassium from a hemolysate. Results indicate that potassium is not bound to hemoglobin since hemoglobin concentration has no effect on the diffusion rate. This suggests that Hb F is not a contributing factor in the ion metabolism of cord erythrocytes.

5. After three weeks storage at 4°C, cord red cells do not differ from adult cells in their ability to resynthesize organic phosphates.

6. With the experimental method described, adenosine causes red cells to lose potassium, gain sodium and shrink in size during storage at 4°C. The

results of Kahn and Cohen indicate that this is caused by the inhibitory effect of adenosine on potassium transport.

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