The Sodium Pump and the Pump-ATPase of Erythrocyte Membranes Prepared from Cold-Stored Blood[1] (36268)

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In red cells the Na^+,K^+-ATPase complex of the membrane utilizes ATP as an energy source to catalyze the coupled translocations of Na^+ and K^+ against the concentration gradients of the ions. This Na^+,K^+-pump is involved in the maintenance of the red cell volume (1, 2), and is an important regulator of the cell metabolism (3). Therefore, the normal operation of the pump is essential to the survival of the red cell.

It has been recognized for years (4) that among the consequences of cold-storage of red cells are: (i) Decrease in ATP content of the cells. (ii) Increase in Na^+ and decrease in K^+ contents due to the reduced activity of the pump at low temperatures. If the stored cells that are introduced into a physiological environment are to remain viable, the activity of the pump must return to normal. For this to occur, not only the supply of the energy source (ATP) for the pump must be replenished, but the enzymic machinery of the pump must have remained sufficiently intact to utilize the energy and move the ions across the membrane. In the past, much work has been done on the nucleotide metabolism of red cells during and after cold storage (4). But to our knowledge no systematic investigation on the behavior of the enzymic pump during storage has been reported. In this paper we present the results of such studies. We stored human blood under conditions that are generally used in blood banks; and after various periods of storage we performed the following experiments: (i) Fragmented membranes were prepared and assayed for Na^+,K^+-ATPase activity. (ii) Washed and resealed ghosts that are deficient in substrates were prepared (Type 1 ghosts); inosine was used as a substrate for lactate production, and active efflux of Na^+ was measured. (iii) Resealed ghosts filled with ATP were made (Type 2 ghosts), and the active efflux of Na^+ was measured in the absence of any other added substrate.

Materials and Methods. Blood was withdrawn from healthy young adults, introduced directly into a blood-bank bottle containing ACD solution, and stored at 4°. Immediately after withdrawal, and at various times during the storage period, samples were removed from the blood for experiments. For some of the enzymic studies, out-dated blood obtained from a blood bank was also used. Fragmented red cell membranes were prepared and assayed for Na^+,K^+-ATPase as described before (5). Procedures for the preparation of Types 1 and 2 ghosts, labeled with ^{22}Na, have been written in detail elsewhere (6–8). In the same references, methods for the determination of Na^+ efflux, and measurements of ATP and ion contents of ghosts have been described. ^{22}Na was obtained from New England Nuclear (Boston, MA). Inosine and ATP were purchased from Sigma Chemical Co. (St. Louis, MO).

Results and Discussion. Red cell membranes from 50 samples of out-dated blood, stored for periods ranging from 21 to 90 days, were prepared and assayed for Na^+,K^+-ATPase activity. The range of activities were 0.8–1.9 μmoles of P_i/ml of ghosts/hr. No correlation between the age of the blood sample and the enzyme activity was observed. Next, the enzyme activity of a blood sample at various times during the storage was determined. The data of Fig. 1 show

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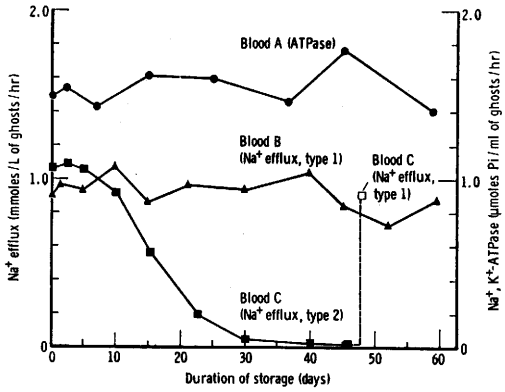

FIG. 1. Blood from three different donors (A, B, C) were collected and used for these studies: (●) ATPase activity of membranes prepared from blood sample A; (▲) Na^+ efflux measured in Type 1 ghosts prepared from blood sample B; (■) Na^+ efflux measured in Type 2 ghosts prepared from blood sample C; (□) Na^+ efflux measured in Type 1 ghosts prepared from blood sample C. ATPase activity refers to that portion of the total enzymic activity that is dependent on the presence of Na^+ and K^+, and can be completely inhibited by 10^{-4} *M* ouabain. All enzyme assays were done in triplicate. Na^+ efflux refers to that portion of the total efflux that is dependent on the presence of K^+ in the medium, and can be completely inhibited in the presence of 10^{-4} *M* ouabain. In each experiment, ^{22}Na released from the ghosts was determined at three different time intervals. Outward rate constant and Na^+ efflux were calculated from these experimental data as described before (7).

that no significant changes in the activity occurred within the tested period of storage. Resealed ghosts were then prepared from two other blood samples after varying periods of storage, and tested for their ability to transport Na^+ actively. Type 1 ghosts were prepared from one sample, and Type 2 ghosts from the other. The results of these experiments, also included in Fig. 1, clearly show that the magnitude of the active efflux of Na^+ from Type 1 ghosts remained constant within the tested period, whereas Type 2 ghosts lost the capacity to pump Na^+ after 3–4 weeks of storage. Two trivial, but possible, explanations for the surprising results obtained with Type 2 ghosts came to mind. First, the enzymic pump in the particular blood sample used for the preparation of Type 2 ghosts may have become accidentally inactivated during the storage. To test this possibility, a portion of this blood, after 45 days of storage, was used to prepare Type 1 ghosts. As shown in Fig. 1 the active efflux of Na^+ in these ghosts was quite normal. Alternatively, it seemed possible that the absence of the pumping activity in Type 2 ghosts could have been due to the loss of the ability of ghosts to retain sufficient amounts of ATP during the resealing procedure. Experiments of Fig. 2 were designed primarily to test this possibility. Two different blood samples were used. Types 1 and 2 ghosts were prepared from each sample at various stages of storage. Prior to the determination of Na^+ efflux, the ATP levels in Type 2 ghosts were also measured. The results demonstrate that, in both blood samples at a time when the pump is still operating in Type 1 ghosts, the active efflux of Na^+ is not observed in Type 2 ghosts in spite of the high levels of ATP within these ghosts.

The posttransfusion viability of erythrocytes is lost after about 3–4 weeks of cold storage (4). The data presented here clearly show that the Na^+, K^+-ATPase of the membranes remains fully active long after the loss of viability. Since the enzymic machinery of the pump is not damaged during the storage, one might have expected the normal

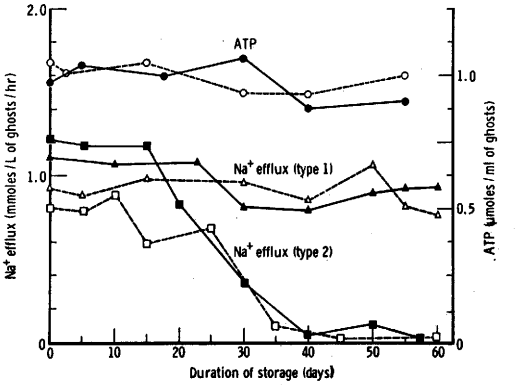

FIG. 2. Two different blood samples [(—) (- - -)] were used in these experiments: Na^+ efflux was determined as described in Fig. 1 legend. Each ATP value is the average of three determinations: Na^+ efflux measured in: (△, ▲) Type 1 ghosts; (□, ■) Type 2 ghosts; (○, ●) ATP content of Type 2 ghosts.

operation of the pump in the ghosts of the stored cells when a source of energy was supplied to the ghosts. However, as the data indicate, after a certain period of storage the enzymic pump can use the ATP that is generated by the metabolism of inosine, but not the ATP that is placed inside the ghosts by the process of resealing. The cause of this phenomenon is not known. But one may speculate that the ATP which is incorporated into the ghosts must be able to penetrate a barrier and approach the enzymic pump within the intact membrane, and that after a period of cold storage this approach (or transport) process becomes inoperative. On the other hand, when inosine is metabolized, ATP may be generated in close proximity to the enzymic pump and utilized by it. The idea that at least a portion of ATP synthesis occurs within the membrane phase of the red cells has been advocated for a long time (9, 10), and has gained further experimental support recently (11).

Regardless of the mechanisms involved, the observations described here are significant in relation to the use of nucleosides as adjuvants added to preservation solutions. It seems that, after a few weeks of cold-storage, the use of inosine is an excellent way of returning the operation of the Na^+,K^+-pump to normal, and that this effect of inosine is not simply due to the restoration of normal ATP levels, but rather due to the generation of a specific energy pool that can be utilized by the pump.

Summary. Blood was stored in ACD solution at 4°. The Na^+,K^+-ATPase activity of the red cell membranes remained constant during prolonged storage (60 days). Within this period the membranes also retained the capacity to transport Na^+ actively when inosine was used as the source of energy for the pump. However, after 3–4 weeks of storage, active transport of Na^+ by the membranes could not be detected when ATP was incorporated into the ghosts. These observations suggest that the ATP generated by the metabolism of inosine may be in a pool that is more suitable for utilization by the pump.

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