Active Calcium and Strontium Transport in Human Erythrocyte Ghosts

ERIK J. OLSON and RALPH J. CAZORT

From the Department of Pharmacology, Meharry Medical College, Nashville, Tennessee 37208

ABSTRACT Both calcium and strontium could be transported actively from erythrocytes if adenosine triphosphate, guanosine triphosphate, or inosine triphosphate were included in the hypotonic medium used to infuse calcium or strontium into the cells. Acetyl phosphate and pyrophosphate were not energy sources for the transport of either ion. Neither calcium nor strontium transport was accompanied by magnesium exchange, and the addition of Mg^{++} to the reaction medium in a final concentration of 3.0 mmoles/liter did not promote the transport of either ion. In the absence of nucleotide triphosphates, the addition of 1.5 mmoles/liter of Sr^{++} to the reaction solution did not bring about active calcium transport and similarly 1.5 mmoles/liter of Ca^{++} did not bring about active strontium transport. The inclusion of 1.5 mmoles/liter of Ca^{++} or Sr^{++} in the reaction medium did not interfere with the transport of the other ion when the erythrocytes were infused with adenosine triphosphate.

The transport of sodium and potassium has received considerable attention in the last decade. In erythrocytes this transport appears to be associated with a membrane ATPase which is activated by sodium, potassium, and magnesium (Dunham and Glynn, 1961; Post et al., 1960). The $Na^+ + K^+ + Mg^{++}$–activated ATPases of erythrocyte membranes (Nakao et al., 1963) and microsomes (Rendi and Uhr, 1964), have been separated from $Ca^{++} + Mg^{++}$–activated ATPases. Wins and Schoffeniels (1966 *a*) investigated the $Ca^{++} + Mg^{++}$ ATPase and found that strontium also could stimulate ATPase activity in these preparations when magnesium was present. Since sodium amytal and dinitrophenol inhibit both $Ca^{++} + Mg^{++}$ and $Sr^{++} + Mg^{++}$ ATPase activity, it is considered that strontium substitutes for calcium in the $Ca^{++} + Mg^{++}$ erythrocyte membrane ATPase.

Recently Schatzmann (1966) demonstrated that intracellular calcium can be transported out of erythrocyte ghosts. The process required adenosine triphosphate but not external magnesium or sodium. Mersalyl inhibited both active calcium transport (Schatzmann, 1966) and the $Ca^{++} + Mg^{++}$–activated ATPase (Vincenzi and Schatzmann, 1967), indicating that the transport of calcium was dependent on the $Ca^{++} + Mg^{++}$–activated ATPase

of erythrocyte membranes. The present investigation sought to determine whether Sr^{++} could be transported from the cells and, if so, what similarities exist between active calcium and active strontium transport.

METHODS

Infusion of Erythrocytes with Calcium or Strontium Human erythrocytes were collected from heparinized blood and washed several times with a solution which contained NaCl in a concentration of 0.13 M and Tris in a concentration of 0.02 M, pH 7.4. Calcium or strontium was then infused into two equal portions of erythrocytes by the method of Schatzmann (1966). The first portion of erythrocytes was exposed to a hypotonic solution which contained 1 mmole/liter of $CaCl_2$ or $SrCl_2$ and 5 mmoles/

TABLE I

RECOVERIES OF CALCIUM, STRONTIUM, AND MAGNESIUM FROM INFUSED RED CELL GHOSTS

Recovery of calcium, strontium, and magnesium after known amounts of these cations were added to infused erythrocyte ghosts prior to digestion with nitric acid. Only one cation was added in each experiment.

Cells infused with	Concentrations		
	Ca	Sr	Mg
		mEq/liter ghosts*	
Ca^{++}	3.2	—	—
Ca^{++} + 2.0 mEq/liter Ca	5.1	—	—
		Recovery 95%	
Sr^{++}	—	2.3	—
Sr^{++} + 2.0 mEq/liter Sr	—	4.5	—
		Recovery 110%	
Ca^{++}	—	—	0.7
Ca^{++} + 2.0 mEq/liter Mg	—	—	2.5
		Recovery 90%	

* In this and the following tables this abbreviation stands for milliequivalents per liter of ghosts.

liter of Tris buffered at pH 7.4. The second portion was exposed to this same hypotonic solution with one of the following additions in mmoles: adenosine triphosphate, 1.65, inosine triphosphate, 1.69, guanosine triphosphate, 1.64, acetyl phosphate, 13.1, or potassium pyrophosphate, 3.13. The cells were then brought back to isotonicity with 3 M KCl. After the infusion, the cells were centrifuged and washed at 0°C in the calcium or strontium reaction mixture which was used in the subsequent incubations.

Incubations of the Infused Cells The first portion of the cells that had been exposed to a hypotonic solution without the added nucleotides, acetyl phosphate, or pyrophosphate was then incubated with an equal volume of a reaction solution containing: NaCl in a concentration of 0.13 M; Tris in a concentration of 0.02 M, pH 7.4; $CaCl_2$ or $SrCl_2$ in a concentration of 1.5 mmoles/liter and 1.0 g/liter of bovine serum albumin. The addition of bovine serum albumin to the reaction mixture minimized the loss of

hemoglobin from the cells during the incubation period. Other aliquots of this portion of the cells were incubated with the same reaction solution plus $MgCl_2$ at a concentration of 3.0 mmoles/liter. The second portion of the cells that had been previously exposed to the nucleotides during the infusion procedure was also incubated with an

TABLE II

THE EFFECT OF MAGNESIUM ON CALCIUM TRANSPORT

Red cell sample	Time	Dry weight	Ca^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*	
			Experiment A			
None	—	—	3.0	—	0.0	—
Control stock	—	22.0	—	3.4	—	0.80
Control	0	22.0	2.6	3.7	0.1	0.80
Control	60	21.0	3.0	3.6	0.2	0.75
None	—	—	3.0	—	0.0	—
ATP stock	—	22.0	—	3.7	—	1.00
ATP	0	23.0	3.1	4.1	0.1	0.95
ATP	30	25.0	4.1	3.3	0.1	1.00
ATP	60	20.0	4.6	2.9	0.1	1.10
None	—	—	3.0	—	2.0	—
ATP	0	22.0	3.6	3.6	1.4	1.40
ATP	30	24.0	4.2	2.7	1.4	1.50
ATP	60	20.5	4.6	2.6	1.3	1.55
			Experiment B			
None	—	—	3.0	—	0.0	—
Control stock	—	15.0	—	2.8	—	0.8
Control	0	13.7	2.8	3.1	0.15	0.7
Control	30	15.0	2.8	3.2	0.10	0.7
Control	60*	14.0	2.8	2.8	0.10	0.7
None	—	—	3.0	—	6.0	—
Control	0	12.8	2.8	3.2	4.7	2.0
Control	30	14.6	2.8	3.2	4.7	2.0
Control	60	13.5	2.8	3.3	4.7	2.4
None	—	—	3.0	—	0.0	—
ATP stock	—	17.0	—	3.0	—	1.0
ATP	0	14.7	3.6	2.5	0.1	0.8
ATP	30	15.9	4.1	2.0	0.1	0.8
ATP	60*	15.4	4.3	2.1	0.1	0.8
None	—	—	3.0	—	1.0	—
ATP	0	14.5	3.6	2.9	0.8	1.0
ATP	30	14.8	4.1	1.8	0.8	1.0
ATP	60	15.0	4.3	2.1	0.8	1.0

* Averages of five runs ATP-60, 4.5, SD ± 0.4, control—60, 3.0 SD ± 0.2. Significance by Student's *t* test $P < 0.01$.

TABLE III

CALCIUM TRANSPORT WITH DIFFERENT HIGH ENERGY PHOSPHATE COMPOUNDS

Red cell sample	Time	Dry weight	Ca^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*	
			Experiment A			
None	—	—	3.0	—	0.0	—
Control stock	—	12.5	—	3.1	—	0.4
Control	0	10.8	3.2	3.7	0.1	0.3
Control	60	11.2	3.2	3.9	0.1	0.3
None	—	—	3.0	—	0.0	—
GTP stock	—	14.0	—	3.7	—	0.7
GTP	0	12.6	3.6	3.6	0.1	0.6
GTP	30	13.3	4.0	—	0.1	0.6
GTP	60	13.7	4.3	2.7	0.1	0.6
None	—	—	3.0	—	1.0	—
GTP	0	13.1	3.5	3.7	0.8	0.8
GTP	30	13.8	4.2	—	0.8	0.8
GTP	60	13.2	4.5	2.6	0.8	0.7
			Experiment B			
None	—	—	3.0	—	0.00	—
Control stock	—	19.4	—	3.0	—	1.05
Control	0	18.0	3.0	3.0	0.05	1.10
Control	60	16.8	3.2	2.8	1.10	1.00
None	—	—	3.0	—	0.00	—
ITP stock	—	17.8	—	3.8	—	1.50
ITP	0	18.1	3.6	3.0	0.05	1.60
ITP	60	18.4	4.4	2.8	0.05	1.30
None	—	—	3.0	—	1.0	—
ITP	0	19.0	3.5	3.0	0.95	1.30
ITP	30	18.3	3.8	—	0.90	1.30
ITP	60	20.1	4.6	2.8	1.10	1.60
			Experiment C			
None	—	—	3.0	—	0.00	—
Control stock	—	14.5	—	3.1	—	0.70
Control	0	11.7	2.9	2.9	0.15	0.80
Control	60	12.2	3.0	3.0	0.15	0.60
None	—	—	3.0	—	0.00	—
ATP stock	—	16.6	—	2.6	—	0.80
ATP	0	13.4	2.7	3.0	0.15	0.80
ATP	60	15.3	4.3	1.9	0.10	0.80
None	—	—	3.0	—	0.00	—
AcP stock	—	13.4	—	2.5	—	0.70
AcP	0	13.1	2.9	2.9	0.10	1.00
AcP	30	14.3	3.0	2.9	0.10	1.00
AcP	60	13.8	3.1	3.1	0.10	0.60
None	—	—	3.0	—	1.00	—
AcP	0	13.0	2.9	3.1	0.80	0.60
AcP	30	13.5	3.0	2.9	0.85	0.80
AcP	60	13.5	3.1	2.9	0.80	1.00

TABLE III—*Concluded*

Red cell sample	Time	Dry weight	Ca^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*	
		Experiment D				
None	—	—	3.0	—	0.0	—
Control stock	—	17.5	—	3.7	—	1.3
Control	0	16.4	3.1	4.0	0.1	1.2
Control	60	18.3	3.0	3.3	0.1	1.1
None	—	—	3.0	—	0.0	—
ATP stock	—	18.3	—	3.8	—	1.2
ATP	0	16.2	4.2	3.7	0.1	1.1
ATP	60	18.1	4.9	2.5	0.1	1.1
None	—	—	3.0	—	0.0	—
Pyrophosphate stock	—	14.8	—	4.0	—	1.1
Pyrophosphate	0	14.2	2.9	4.4	0.1	1.0
Pyrophosphate	30	15.0	2.6	4.8	0.1	1.1
Pyrophosphate	60	15.8	2.7	4.6	0.1	1.1
None	—	—	3.0	—	1.0	—
Pyrophosphate	0	14.4	2.5	4.4	0.7	1.3
Pyrophosphate	30	14.6	2.3	4.6	0.7	1.5
Pyrophosphate	60	15.8	2.4	5.2	0.7	1.7

equal volume of the reaction solution or the same reaction solution containing $MgCl_2$ in a concentration of 0.5 to 1.0 mmoles/liter. Some of the experiments with Ca^{++}-infused cells employed reaction solutions containing 1.5 mmoles/liter of $SrCl_2$ while some experiments with Sr^{++}-infused cells employed reaction solutions which contained 1.5 mmoles/liter of $CaCl_2$.

Except in experiments in which acetyl phosphate was infused into cells, the cells were equilibrated at 37°C for 10 min before suspension in the reaction solution. This step was omitted for acetyl phosphate to minimize its possible breakdown by a K^+ stimulated acyl phosphatase in the erythrocyte membrane (Bader and Sen, 1966; Isreal and Titus, 1967; Sachs et al., 1967). At the prescribed time intervals the cells were separated from the supernatant by centrifugation. The cells and supernatants were then subjected to appropriate analysis.

Dry Weight Determinations Aliquots of the infused cells were added to weighed vials, heated for 24 hr at 100°C, and weighed again.

Calcium, Strontium, and Magnesium Analysis of Infused Cells and Supernatants The cells were digested at room temperature in 3 volumes of concentrated nitric acid for a period of several days. The digest was then evaporated to dryness and taken up in 3 volumes of 1 M HCl, and analyzed for calcium, magnesium, and strontium by atomic absorption spectroscopy (Dawson and Heaton, 1961). Undigested supernatants were also analyzed for these ions by atomic absorption spectroscopy. All samples were diluted with 0.5% lanthanum chloride.

Recoveries Known amounts of calcium and strontium were added to one of two equal aliquots of infused cells and, after digestion, the increase in the particular ion was noted and compared with the predicted value.

Chemicals Adenosine triphosphate, inosine triphosphate, and guanosine triphosphate were obtained as their sodium salts from Nutritional Biochemicals Corp. (Cleveland, Ohio). Dilithium acetyl phosphate was obtained from Sigma Chemical Company (St. Louis, Mo.). Bovine serum albumin was obtained from Armour, Inc. (Chicago, Ill.).

RESULTS

General Considerations It can be seen from Tables II through VII that calcium and strontium were transported out of the ghosts against moderate gradients and that, within the limits of experimental error, there was agreement between the increase in calcium and strontium in the supernatant and the decrease in the concentrations of these ions in the ghosts. Dry weight analysis showed that the observed concentration changes were not due to water entering the cells during the incubation (Tables II–VII). The variation in the dry weights between individual experiments was probably due to variations in the time of the hypotonic exposure period for the infusion of calcium or strontium into the cells. The tables represent typical experiments. Most experiments were performed three times while experiment B in Table II was performed five times. Active transport of calcium and strontium was always observed in the presence of a nucleotide triphosphate. The recoveries of added calcium, strontium, and magnesium were checked twice and found to be near 100% (Table I).

Magnesium and Active Calcium Transport It is apparent that calcium was transported from the cells without a significant uptake of magnesium when magnesium ions were present at concentrations of 0.5 mmole/liter or 1 mmole/liter in the external medium (Table II). Furthermore, in the same medium Mg^{++} in a concentration of 3 mmoles/liter failed to bring about calcium transport in cells that were not infused with ATP.

Specificity The data shown in Table III indicate that inosine triphosphate and guanosine triphosphate can support calcium transport as well as adenosine triphosphate can. The transport observed with these nucleotides also occurred without the uptake of magnesium. Acetyl phosphate was not an energy source for calcium transport. The transport usually observed with ATP at zero time did not occur when the equilibration period was eliminated as in experiment C (Table III). Evidently some transport occurred when the infused cells were incubated for 10 min at 37°C and when the cells were centrifuged from the reaction solution.

The failure of pyrophosphate and acetyl phosphate to induce active

calcium transport indicated that the transport of calcium was not due to the diffusion of phosphate compounds from the erythrocyte ghosts.

Strontium Transport The active transport of strontium is similar in some respects to the active transport of calcium. For example, strontium is removed from the cells without the exchange of magnesium and it cannot be removed from the cell by the passive diffusion of magnesium into the cell (Table IV). As with calcium transport, inosine and guanosine triphosphates

TABLE IV

THE EFFECT OF MAGNESIUM ON STRONTIUM TRANSPORT

Red cell sample	Time	Dry weight	Sr^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*	
None	—	—	3.0	—	0.00	—
Control stock	—	12.4	—	2.5	—	0.6
Control	0	9.7	2.9	3.2	0.10	0.5
Control	30	11.6	2.9	2.6	0.08	0.5
Control	60	12.1	3.0	2.7	0.08	0.5
None	—	—	3.0	—	6.0	—
Control	0	9.9	3.0	2.9	4.6	2.4
Control	30	11.6	3.0	2.8	4.4	2.1
Control	60	12.2	3.2	2.7	4.6	2.0
None	—	—	3.0	—	0.00	—
ATP stock	—	11.9	—	3.0	—	0.7
ATP	0	9.6	4.2	2.7	0.10	0.6
ATP	30	10.6	4.8	2.2	0.05	0.7
ATP	60	11.7	4.9	1.6	0.05	0.7
None	—	—	3.0	—	1.00	—
ATP	0	9.4	4.3	2.6	0.85	0.9
ATP	30	10.2	4.8	2.0	0.85	0.8
ATP	60	11.2	4.8	1.6	0.80	0.9

can serve as well as adenosine triphosphate for active strontium transport (Table V). It may also be noted, that neither acetyl phosphate nor pyrophosphate can serve as an energy source for strontium transport.

Calcium and Strontium Interactions It is evident from Tables VI and VII that both calcium and strontium transport proceed without interference from strontium and calcium, respectively. In these experiments there was virtually no movement of magnesium. It is also apparent, that external strontium cannot induce calcium transport and that external calcium cannot induce strontium transport.

TABLE V

STRONTIUM TRANSPORT WITH DIFFERENT HIGH ENERGY PHOSPHATE COMPOUNDS

Red cell sample	Time	Dry weight	Sr^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*	
		Experiment A				
None	—	—	3.0	—	0.00	—
Control stock	—	12.5	—	2.5	—	2.5
Control	0	11.1	2.9	2.5	0.04	0.6
Control	30	12.5	3.0	2.2	0.04	0.4
Control	60	12.6	2.9	2.7	0.05	0.6
None	—	—	3.0	—	0.00	—
GTP stock	—	11.7	—	3.1	—	0.6
GTP	0	9.8	4.0	1.9	0.025	0.4
GTP	30	11.3	4.5	1.8	0.025	0.5
GTP	60	10.1	4.7	1.9	0.025	0.6
None	—	—	3.0	—	1.0	—
GTP	0	10.4	4.2	2.3	0.8	0.9
GTP	30	10.4	4.5	1.7	0.75	0.8
GTP	60	11.5	4.5	1.9	0.80	1.0
		Experiment B				
None	—	—	3.0	—	0.00	—
Control Stock	—	14.3	—	2.8	—	0.8
Control	0	13.0	2.9	3.1	0.04	0.6
Control	60	15.3	3.1	2.7	0.05	0.8
None	—	—	3.0	—	0.00	—
ITP stock	—	11.9	—	2.4	—	0.6
ITP	0	10.8	3.8	1.9	0.02	1.0
ITP	30	11.3	3.9	1.7	0.02	1.0
ITP	60	11.2	4.0	1.9	0.04	1.1
None	—	—	3.0	—	1.00	—
ITP	0	11.2	3.6	2.6	0.85	1.2
ITP	30	11.4	3.9	2.0	0.80	0.9
ITP	60	11.4	4.0	1.7	0.80	1.3
		Experiment C				
None	—	—	3.0	—	0.00	—
Control stock	—	14.6	—	2.7	—	0.5
Control	0	12.4	2.9	2.9	0.15	0.4
Control	60	13.0	3.0	2.7	0.20	0.4
None	—	—	3.0	—	0.00	—
ATP stock	—	13.8	—	2.7	—	0.6
ATP	0	10.8	2.9	3.5	0.10	0.5
ATP	60	12.4	5.0	2.0	0.10	0.5
None	—	—	3.0	—	0.00	—
AcP stock	—	13.1	—	2.5	—	0.7
AcP	0	12.1	2.9	2.2	0.10	0.5
AcP	30	13.6	3.0	2.4	0.10	0.6
AcP	60	13.0	3.0	2.4	0.10	0.5

TABLE V—*Concluded*

Red cell sample	Time	Dry weight	Sr^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*	
None	—	—	3.0	—	1.0	—
AcP	0	12.0	2.9	2.0	0.80	0.8
AcP	30	13.8	3.0	2.7	0.80	0.8
AcP	60	12.8	3.0	2.3	0.80	0.8
		Experiment D				
None	—	—	3.0	—	0.0	—
Control stock	—	14.3	—	3.1	—	0.9
Control	0	13.8	2.8	3.9	0.10	0.8
Control	60	14.2	3.1	3.5	0.15	0.7
None	—	—	3.0	—	0.00	—
ATP stock	—	15.0	—	3.5	—	1.1
ATP	0	14.0	4.0	3.3	0.10	0.9
ATP	60	12.9	5.2	2.5	0.15	0.9
None	—	—	3.0	—	0.00	—
Pyrophosphate stock	—	10.0	—	4.4	—	0.6
Pyrophosphate	0	10.6	2.7	3.8	0.10	0.5
Pyrophosphate	30	11.5	2.7	4.4	0.10	0.9
Pyrophosphate	60	9.8	2.7	4.4	0.15	0.7
None	—	—	3.0	—	1.0	—
Pyrophosphate	0	9.8	2.7	4.7	0.65	1.1
Pyrophosphate	30	9.3	2.7	4.4	0.65	1.1
Pyrophosphate	60	8.8	2.7	4.6	0.65	1.1

DISCUSSION

Under our experimental conditions the calcium and strontium transport system functions at internal magnesium concentrations that are well below the observed Michaelis constant for the enzyme (Wins and Schoffeniels, 1966 *a*). It is possible that a significant portion of the magnesium remaining in the cell became bound to the membrane during the calcium infusion procedure so that it was concentrated near the Ca^{++} + Mg^{++}–stimulated ATPase of the membrane.

Magnesium leakage from the cells was minimal and the calcium transport system functioned at external magnesium concentrations of 0.05 mmole/liter or less. In accord with the previous findings of Schatzmann (1967) added magnesium was not taken up by cells which transport calcium. It is, therefore, very unlikely that magnesium exchange is a necessary or supplemental feature of the calcium transport system. In this regard the calcium transport system is different from the active sodium transport system in which there is an exchange of intracellular sodium for extracellular potassium (Post and Jolly, 1957; Garrahan and Glynn, 1967).

TABLE VI

EFFECT OF STRONTIUM ON CALCIUM TRANSPORT

Rell cell sample	Time	Dry weight	Ca^{++} concentration supernatant cells		Sr^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*		*mEq/liter*	
None	—		3.0	—	0.0	—	0.0	—
Control stock	—	14.0	—	2.9	—	—	—	0.7
Control	0	12.2	3.0	2.9	—	—	0.2	0.8
Control	60	12.7	3.0	3.0	—	—	0.1	0.7
None	—		3.0	—	3.0	—	0.0	—
Control	0	12.6	3.0	3.0	2.6	0.6	0.1	0.6
Control	60	13.0	3.1	3.0	2.3	0.7	0.2	0.6
None	—		3.0	—	0.0	—	0.0	—
ATP stock	—	13.8	—	3.0	—	—	—	0.6
ATP	0	12.0	3.5	3.0	—	—	0.1	0.6
ATP	30	12.5	4.2	2.7	—	—	0.1	0.6
ATP	60	13.2	4.7	2.1	—	—	0.1	0.7
None	—		3.0	—	3.0	—	0.0	—
ATP	0	14.4	3.5	2.9	2.3	0.7	0.1	0.7
ATP	30	13.0	4.2	2.6	2.6	0.6	0.1	0.7
ATP	60	13.4	4.7	2.1	2.3	0.6	0.1	0.7

TABLE VII

THE EFFECT OF CALCIUM ON STRONTIUM TRANSPORT

Rell cell sample	Time	Dry weight	Sr^{++} concentration supernatant cells		Ca^{++} concentration supernatant cells		Mg^{++} concentration supernatant cells	
	min	%	*mEq/liter*		*mEq/liter*		*mEq/liter*	
None	—	–	3.0	—	0.0	—	0.0	—
Control stock	—	13.2	—	2.7	—	—	—	0.6
Control	0	13.0	2.6	3.0	—	—	0.2	0.6
Control	30	14.7	2.6	3.2	—	—	0.2	0.6
Control	60	12.7	2.9	2.8	—	—	0.2	0.6
None	—	—	3.0	—	3.0	—	0.0	—
Control	0	12.7	2.7	2.8	1.9	1.4	0.2	0.6
Control	30	14.0	2.8	2.8	2.2	1.2	0.2	0.6
Control	60	14.0	2.8	2.9	1.9	1.3	0.2	0.6
None	—	—	3.0	—	0.0	—	0.0	—
ATP stock	—	13.0	—	2.6	—	—	—	0.8
ATP	0	12.7	3.7	2.2	—	—	0.1	0.7
ATP	30	13.5	4.2	1.8	—	—	0.1	0.8
ATP	60	14.2	4.4	1.4	—	—	0.1	0.8
None	—	—	3.0	—	3.0	—	0.0	—
ATP	0	13.1	3.7	2.1	2.3	1.0	0.1	0.7
ATP	30	13.6	4.0	1.9	2.2	1.1	0.1	0.7
ATP	60	13.8	4.2	1.7	2.3	0.9	0.1	0.7

Since no exchange was evident with active calcium transport, one might speculate that a nonspecific exchange with a monovalent cation or the extrusion of an anionic carrier might accompany the removal of intracellular calcium. For example, aspartic acid release accompanied the transport of sodium from the crab nerve in a potassium-free medium (Baker, 1964). External potassium reduced this release of aspartic acid markedly. Perhaps aspartic acid served as a carrier for sodium when it was transported into a potassium-free medium.

Since ITP and GTP can substitute effectively for ATP in the active transport of calcium, it can be speculated that a membrane adenosine triphosphatase, which is activated by calcium and magnesium, can use ITP and GTP as well. The membrane $Ca^{++} + Mg^{++}$–activated adenosine triphosphate may either use ITP and GTP as such, or the terminal phosphate of GTP and ITP may be transferred to enzymatically bound adenosine diphosphate.

It has been found that the $Na^{+} + K^{+} + Mg^{++}$–stimulated adenosine triphosphatase of microsomes also has K^{+}–stimulated acyl phosphatase activity (Bader and Sen, 1966; Isreal and Titus, 1967; Sachs et al., 1967). A small but significant background of acyl phosphatase activity was found in the absence of potassium (Bader and Sen, 1966). It is possible that the residual activity may be related to the calcium transport system. Although this possibility exists, acetyl phosphate was totally ineffective as an energy source for active calcium transport. However, a significant proportion of the infused acetyl phosphate may have been hydrolyzed by K^{+}–stimulated acetyl phosphatase in the membrane. Furthermore, other compounds such as adenosine diphosphate, which may be necessary for the functioning of the pump, were not present.

The similarity of the calcium and strontium transport systems, and the fac that strontium can substitute for calcium with the $Ca^{++} + Mg^{++}$–activated adenosine triphosphatase of the erythrocyte membrane suggest that the two ions use the same transport system. The calcium ion has an atomic radius o 0.98 A while the strontium ion has an atomic radius of 1.15 A (Glasstone, 1940). Both ions have coordination numbers of six. The d orbitals of strontium do not prevent its transport.

At the present time it is not known whether the active transport process is related in any way to the contraction of erythrocyte ghosts observed in the presence of ATP and calcium (Wins and Schoffeniels, 1966 *b*). Our dry weight analysis suggests that the two processes are unrelated.

It has also been noted that the addition of calcium at the time of hemolysis increased the permeability of the ghost membrane to potassium (Hoffman, 1962). Calcium infusion into erythrocytes could also alter the calcium transport system.

The authors are indebted to Dr. William W. Lacy of the Department of Medicine at Vanderbilt University School of Medicine for the use of his atomic absorption spectrometer. The authors would also like to thank Dr. Jane Harting Park of the Department of Physiology at Vanderbilt University School of Medicine and Dr. Charlotte R. Hollett of the Department of Pharmacology at Meharry Medical College for their suggestions concerning the preparation of this manuscript.

This work was supported by an intramural grant (No. 5-SO-IFR-05422-07) from the National Institutes of Health, United States Public Health Service.

Received for publication 21 May 1968.

REFERENCES

BADER, H., and A. K. SEN. 1966. K^+-dependent acylphosphatase as part of the $Na^+ + K^+$ dependent ATPase of cell membranes. *Biochim. Biophys. Acta.* **118**:116.

BAKER, P. F. 1964. An efflux of ninhydrin positive material associated with the operation of Na^+ pump in intact crab nerve immersed in Na^+ free solutions. *Biochim. Biophys. Acta.* **88**:458.

DAWSON, J. B., and J. W. HEATON. 1961. Determination of magnesium in biological materials by atomic absorption spectrometry. *Biochem. J.* **80**:99.

DUNHAM, E. T., and I. M. GLYNN. 1961. Adenosine triphosphatase activity and the active movements of alkali metal ions. *J. Physiol. (London).* **156**:274.

GARRAHAN, P. J., and I. M. GLYNN. 1967. The stoichiometry of the sodium pump. *J. Physiol. (London).* **192**:217.

GLASSTONE, S. 1940. Textbook of Physical Chemistry. D. Van Nostrand Co., Inc., New York. p. 375.

HOFFMAN, J. F. 1962. Cation transport and structure of the red cell membrane. *Circulation.* **26**:1201.

ISREAL, Y., and E. TITUS. 1967. A comparison of microsomal ($Na^+ + K^+$) ATPase with K acetyl phosphatase. *Biochim. Biophys. Acta.* **139**:450.

NAKAO, T., K. NAGANO, K. ADACHI, and M. NAKAO. 1963. Separation of two adenosine triphosphatases from erythrocyte membranes. *Biochim. Biophys. Res. Commun.* **13**:444.

POST, R. L., and P. C. JOLLY. 1957. The linkage of Na^+, K^+ and ammonium active transport across the human erythrocyte membrane. *Biochim. Biophys. Acta.* **25**:118.

POST, R. L., C. R. MERRITT, C. R. KINSOLVING, and C. D. ALBRIGHT. 1960. Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. *J. Biol. Chem.* **235**:1796.

RENDI, R., and M. L. UHR. 1964. Sodium, potassium requiring adenosine triphosphatase activity; purification and properties. *Biochim. Biophys. Acta.* **89**:520.

SACHS, G., J. D. ROSE, and B. I. HIRSCHOWITZ. 1967. Acetyl phosphatase in brain microsomes: A partial reaction of $Na^+ + K^+$ ATPase. *Arch. Biochem. Biophys.* **119**:277.

SCHATZMANN, H. J. 1966. ATP-dependent Ca^{++}-extrusion from human red cells. *Experientia (Basel).* **22**:364.

SCHATZMANN, H. J. 1967. Ca-activated ATPase in human red cells and its possible role in active calcium transport. *Protides Biol. Fluids. Proc. Colloq. Bruges.* **15**:251.

VINCENZI, F. F., and H. J. SCHATZMANN. 1967. Some properties of Ca-activated ATPase in human red cell membranes. *Helv. Physiol. Pharmacol. Acta.* **25**:Cr. 233.

WINS, P., and E. SCHOFFENIELS. 1966 *a*. Studies on red cell ghost ATPase systems: Properties of a ($Mg^{++} + Ca^{++}$) dependent ATPase. *Biochim. Biophys. Acta.* **120**:341.

WINS, P., and E. SCHOFFENIELS. 1966 *b*. ATP + Ca^{++}-linked contraction of red cell ghosts. *Arch. Int. Physiol. Biochem.* **74**:812.