THE LINKAGE OF SODIUM, POTASSIUM, AND AMMONIUM ACTIVE TRANSPORT ACROSS THE HUMAN ERYTHROCYTE MEMBRANE*

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It is well established that human erythrocytes actively transport potassium inward and sodium outward across their membranes against electrochemical potential gradients with energy from glycolysis. There is good evidence that these ions also diffuse through the membrane passively by a path which is independent of and in parallel with that of active transport. Almost all intracellular sodium and potassium appear to be free in solution. TOSTESON[1] has reviewed the subject recently. It has also been demonstrated that there is an interdependence between the active transports of potassium and sodium. In 1949 FLYNN AND MAIZELS[2] reported a slowing of potassium transport inward with lowered internal sodium concentrations and a slowing of sodium transport outward with lowered external potassium concentrations. HARRIS AND MAIZELS[3] confirmed the latter observation using radioactive sodium and presented evidence[4] that any linkage between the two systems cannot be electrostatic. They suggested that "inward transport of K is "geared" to outward Na transport by the use of a common carrier". Both authors have presented detailed theories of linkage[5,6]. HARRIS proposed that the transports are numerically related with one potassium atom being transported inward for every two sodium atoms transported outward. GLYNN[7] observed parallel changes in the active fluxes of both cations as the external potassium concentration was varied.

This paper will present evidence that the active transports of potassium and sodium are rigidly linked in a ratio of two atoms of potassium to three atoms of sodium. The evidence is as follows. 1. Reciprocal dependence of transport is complete; that is, either the absence of external potassium or the absence of internal sodium stops the active transport of both ions. 2. Two atoms of potassium are transported inward for every three atoms of sodium transported outward over a wide range of conditions. 3. Such a linkage makes it possible to interpret semiquantitatively the effects of ammonium as due to the direct substitution of ammonium for potassium. Part of this material has been presented in preliminary form[8,9].

METHODS

Outline

Human erythrocytes were prepared by allowing them to fill with sodium and empty of potassium during storage at 2° in a sodium medium for various periods up to 5½ weeks. Transport was observed during incubation at 37° by determining the cation contents of the cells at different times

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for up to 8 hours. Active transport was distinguished from passive transport by adjusting the external sodium and potassium concentrations to minimize net passive transport and by observing control cells treated with strophanthin[10].

Media

The composition of a representative cold storage medium is given below.

	mmoles/l		mmoles/l
NaCl	110	Adenosine	3.7
Na_2HPO_4	25	Glucose	10
HCl	2	Bovine albumin 1 g/l	- -
$MgCl_2$	2		

The composition of incubation media was similar except that some or all of the sodium was replaced by potassium. Adenosine was added to prevent the usual deterioration of active transport which occurs during cold storage[11]. Adenosine was replaced with inosine in experiments with low extracellular potassium, because the ammonia liberated from adenosine by nucleoside deaminase was found to affect the results. Magnesium was added because many red cell enzymes require it, but good transport has been obtained even in a medium containing 50 m*M* ethylenediaminetetraacetate, a cation-chelating agent. The presence or absence of 2 mmoles/l of calcium did not affect the results. Bovine serum albumin reduced the small amount of hemolysis which was usually present and did not affect transport. Chloride has been replaced by nitrate, and phosphate buffer has been replaced by glycyl-glycine buffer without affecting transport.

Preparation of the cells

The duration of the cold storage period varied according to the initial cation contents desired for incubation. The cold storage medium was renewed every two to four days to provide fresh substrate and to keep the pH at 7.4 ± 0.4. Before incubation the cells were filtered and packed and the top layer was discarded. The remaining cells were stirred thoroughly and 0.4 to 0.8 ml aliquots were washed twice at room temperature with 30 ml of incubation medium. The hematocrit of the incubated suspension was usually 2 to 5 %.

Analysis of the cells

The cells were washed free of incubation medium and a hemolysate was analysed for sodium, potassium and hemoglobin. The cation contents of the cells were determined by the ratio of the cation concentrations to the hemoglobin concentration. In particular, the aliquots of incubated cell suspension were cooled immediately and washed twice with 50 to 100 times their volume of isotonic choline chloride at 2° by centrifugation and resuspension. The cells lost less than 1 % of their internal cations in this process. The rest of the procedure was carried out at room temperature. The 0.1 ml samples of washed cells were diluted with about 10 ml of distilled water containing 0.2 % of concentrated ammonia solution and 0.02% of Sterox SE*, a non-ionic detergent. The clear hemolysate thus produced was mixed thoroughly and one aliquot was taken for the determination of hemoglobin by the method of CROSBY *et al.*[12]. The optical density of a solution containing 10 μmoles/l of cyanmethemoglobin was taken as 0.460 at 540 mμ[13]. Another aliquot was analysed directly for sodium and potassium with the hydrogen flame attachment to the Beckman Model DU spectrophotometer. The flame of the photometer was adjusted each day so that the meter reading at 0.5 and 1.0 mequiv./l fitted a previously determined calibration curve. There was no detectable radiation interference between sodium and potassium. Hemoglobin at the concentration present in the samples, about 0.3 %, probably did not produce significant radiation interference, since adding 0.2 to 0.3 % of albumin to standard solutions produced no effect on emission. Sterox SE (0.02 %) increased the aspiration rate and was added to the standard solutions. The results of the analyses are expressed as mequiv. of cation per 5 mmoles of hemoglobin (abbreviated "Hgb") since one liter of normal cells contains about 5 mmoles of hemoglobin. The precision of the analyses ranged from a standard deviation of about 2 mequiv./5 mmoles Hgb in the earlier experiments to 0.8 mequiv./5 mmoles Hgb in the later ones. This fairly simple method of red cell analysis has some advantages. It measures the cation contents directly and does not require correction for interstitial fluid or for cell volume changes which may occur during an experiment.

Errors due to passive transport

Because sodium and potassium move across the membrane by passive diffusion as well as by active transport, it is necessary to take account of passive transport when using net transport to measure active transport. In these experiments three procedures were used to minimize the effects of passive transport. First, adenosine, a metabolic adjuvant[11], was added to accelerate the active rate. Second,

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the external concentrations were adjusted to be less than 60 mequiv./l away from concentrations in equilibrium with the internal contents, so that passive influx and outflux were at least partly equalized. Third, in some experiments passive transport was determined separately in a control flask to which strophanthin was added. SCHATZMANN[10] has shown that strophanthin stops active transport without affecting passive transport or metabolism. In those experiments in which only the first two methods were used it is likely that there was a significant amount of passive transport in some cases. We have estimated the maximum passive transport for a gradient of 60 mequiv./l away from equilibrium* at 1.0 mequiv./5 mmoles Hgb/h from observations on cold-stored cells treated with strophanthin. With active transport at 5 mequiv./5 mmoles Hgb/h this leak corresponds to an estimated maximum error of 20 %. However, even in these cases the error in the ratio of the transport rates was less than that of the individual rates. This is because external concentrations of sodium and potassium which increased the passive transport of one ion in one direction also increased the passive transport of the other ion in the other direction. The passive transport errors were therefore of the same sign and tended to cancel each other in the ratio.

RESULTS

1. *Reciprocal dependence*

Active sodium transport did not take place in the absence of external potassium. Low potassium high sodium cells incubated without external potassium showed no measurable active transport of either ion. When external potassium was added, active

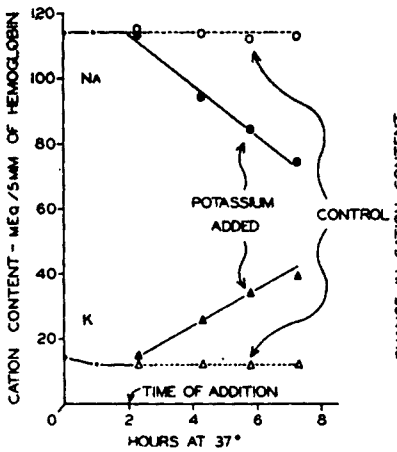

Fig. 1. The absence of active cation transport in human erythrocytes lacking external potassium. The cells were prepared by storage at 2° for 9 days in a sodium medium. They were then incubated at 37°. At first the incubation medium contained only 150 mequiv./l of sodium and no potassium. After 2 hours, as shown by an arrow, the suspension was divided into two parts and enough isotonic KCl (solid symbols) or NaCl (open symbols) was added to each part to have it contribute 21 mequiv./l to the medium. Inosine was used in place of adenosine as a metabolic adjuvant. pH 7.4 ± 0.1 ('56.6.15).

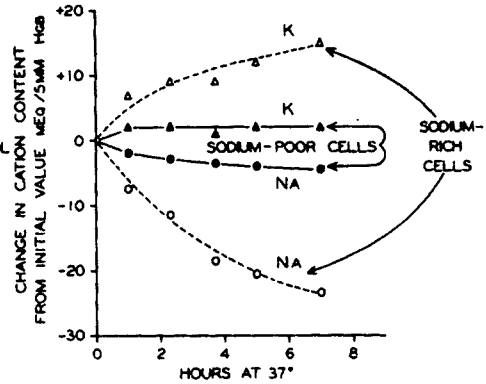

Fig. 2. The depression of active potassium transport in human erythrocytes with low internal sodium. Two lots of cells were prepared by storage at 2° for two days. The control cells were stored in 140 mequiv./l of sodium and 20 mequiv./l of potassium. They contained initially Na = 32 and K = 83 mequiv./5 mmoles Hgb. The test cells were stored similarly except that sodium was replaced by choline. They contained initially Na = 6.6 and K = 83 mequiv./5 mmoles Hgb. Both lots of cells were incubated at 37° in the same medium, which contained 50 mequiv./l of potassium and 110 mequiv./l of choline, in order to minimize net passive potassium transport and to prevent any sodium influx. The changes in the cation contents of the sodium-rich cells are shown by open symbols and those of the sodium-poor cells by solid symbols. pH 7.3 ± 0.2 ('56.0.20)

* For a monovalent cation at equilibrium across the membrane of a normal erythrocyte it was assumed that the external concentration in mequiv./l equals the internal content in mequiv./5 mmoles Hgb. This was on the basis that the action of the membrane potential and the displacement of intracellular water by hemoglobin just cancel each other.

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transport of potassium inward and sodium outward started immediately. The results are shown in Fig. 1. Similarly, active potassium transport did not take place when the internal sodium content was low. Cells were prepared containing different amounts of sodium and the same amount of potassium. They were incubated in a medium containing potassium and choline but no sodium. The high sodium cells showed good active transport of both cations but the low sodium cells took up correspondingly little potassium. The results are shown in Fig. 2. This effect was also observed in cells which were not exposed to choline (see Fig. 6). The potassium uptake of the high sodium cells shows that the internal potassium concentration was not limiting transport. These results extend the original observations of FLYNN AND MAIZELS[2].

2. Stoichiometry

There was a close correlation between the amount of sodium actively transported outward and the amount of potassium actively transported inward. The cells initially contained mostly sodium and different rates of transport were obtained by varying the external potassium concentration. The ratio of transport rates was two atoms of potassium to three of sodium and seemed to persist even at low rates where measurement was not so precise. The results are shown in Fig. 3. The relationship between the rate of transport and the external potassium concentration is shown in Fig. 4. Transport was half-maximal at about 2.2 mequiv./l. The same amount of internal sodium was available in all cases.

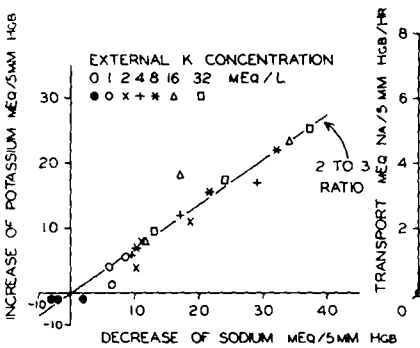

Fig. 3. Simultaneous changes in the cation content of human erythrocytes during active transport at low external potassium concentrations. The cells were prepared by storage in a sodium medium at 2° for 15 days and contained initially Na = 118, K = 9 mequiv./5 mmoles Hgb. During incubation at 37° the (Na + K) content of the medium was 179 mequiv./l in all cases. The initial external potassium concentrations are shown on the graph. Samples were taken at 0, 2, 4, and 6 hours after the onset of incubation. Inosine was used in the place of adenosine as a metabolic adjuvant. pH 7.5 ± 0.1 ('56.6.12).

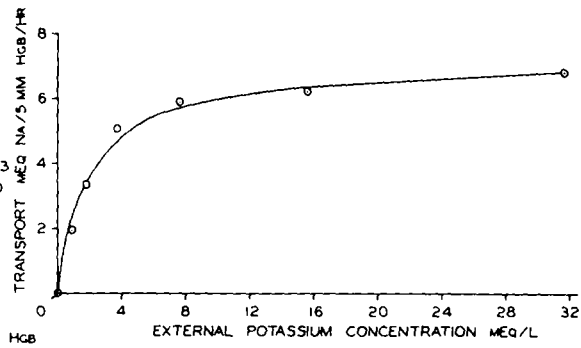

Fig. 4. The influence of the external potassium concentration on the rate of active cation transport in human erythrocytes. The data are the same as those of Fig. 3. Although the rate is expressed in terms of sodium, it was calculated from the data on both cations by means of a 2:3 weighted average. The four points in Fig. 3 with the greatest deviation from the 2 to 3 ratio were not used. The small leak at zero external potassium was added to all the transport rates as a partial passive transport correction. The smooth curve is drawn from the best-fitting Michaelis-Menten equation where the maximal rate, V_{max}, is 7.1 mequiv./5 mmoles Hgb/h and the concentration of potassium at half-maximal transport, K_m, is 2.2 mequiv./l. The equation is $V = \frac{V_{max}[K]}{[K] + K_m}$ where V is the rate and [K] is the potassium concentration.

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The two to three ratio of transport rates was tested again in seven experiments in which different rates of transport were obtained in association with different cell sodium contents. The external potassium concentration was greater than 30 mequiv./l in all cases and was therefore not significantly rate-limiting. Fig. 5 shows that the same ratio was found as before. The rates of transport and corresponding cell sodium contents are shown in Fig. 6. In these experiments the transport rate was not so consistently related to the cell sodium content as it was to the external potassium concentration in the previous experiments. One reason is that the different sodium contents were obtained by storing different lots of cells for different periods of time. The metabolic rates of the different lots of cells undoubtedly varied. The cell sodium contents also varied during the experiments and transport rates had to be calculated from fewer observations over shorter periods of time, so that observational errors were greater. The failure of active potassium transport in the absence of internal sodium shown in Fig. 2 is confirmed here with cells which were not exposed to choline.

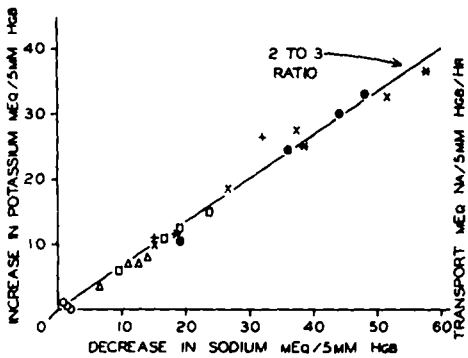

Fig. 5. Simultaneous changes in the cation contents of human erythrocytes during active transport at high external potassium concentrations. The cells were prepared by storage at 2° in a potassium and/or sodium medium for 4 to 25 days in seven experiments. They contained initially Na = 5 to 122 and K = 104 to 11 mequiv./5 mmoles Hgb. During incubation at 37° the external medium contained Na = 0 to 123 and K = 145 to 30 mequiv./l and the cation concentrations were always within ± 60 mequiv./l of the corresponding cell contents. Samples were taken at 1 ½ or 2 hour intervals for up to 6½ hours. The different symbols indicate the different experiments. The corresponding transport rates and cell sodium contents are shown in Fig. 6. The values shown by open symbols have been corrected for passive transport by comparison with paired controls treated with strophanthin. pH 7.1 to 7.8 ('55.0.31 to '56.N.02).

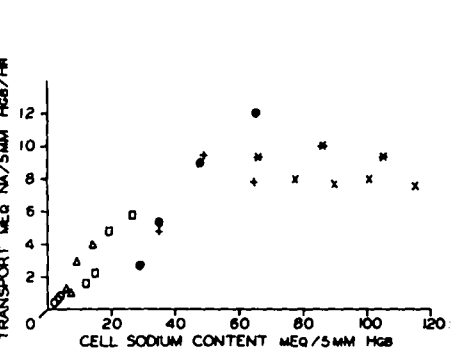

Fig. 6. The influence of cell sodium content on the rate of active cation transport at high external potassium concentrations in human erythrocytes. The data are from the same seven experiments shown in Fig. 5. The different symbols represent the different experiments and correspond to those of Fig. 5. Although the rate is expressed in terms of sodium, it was calculated from the data on both cations by means of a 2:3 weighted average. The values shown by open symbols have been corrected for passive transport by comparison with paired controls treated with strophanthin.

In order to check the previous observations and in order to test the effect of varying the external cation concentrations more widely, the experiments shown in Table I were performed. In these experiments passive transport was measured in one flask to which strophanthin was added and from these results the passive transport present in the other flasks was calculated. The mean value of 0.66 for the ratio of the potassium to sodium active transport rates confirms the value of 2/3 found in the first experiments. Statistical analysis showed that the variation of the ratio from

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TABLE I

THE RATIO OF POTASSIUM TO SODIUM ACTIVE TRANSPORT CORRECTED FOR PASSIVE TRANSPORT WITH STROPHANTHIN

Cells were stored at 2° for 6 to 12 days and contained Na = 77 to 107 and K = 41 to 17 mequiv./5 mmoles Hgb, respectively. They were incubated at 37° in four flasks for 4 or 5 hours. The composition of the medium in flasks, A, B, and C is given in the table. Flask D was the same as C but in addition contained strophanthin-*k* N.F. (5 mg/l). Chloride and water contents of the cells and passive ion movements were measured in flask D. From these measurements the passive ion movements in the other flasks were calculated as follows. The chloride and water contents were used to calculate a membrane factor by which a given cell cation content should be multiplied to obtain the external cation concentration which would be in thermodynamic equilibrium with it. The passive permeability of the cell membrane was then taken as proportional to the ratio of the rate of ion movement in flask D divided by the difference between the actual external ion concentration and the mean external concentration which would have been in equilibrium with the cell ion content. The membrane factor and passive permeability were then used to calculate the passive ion movements in the other flasks. The procedure is equivalent to that of HARRIS[6]. ('56.8.08 to .20.)

Experiment	*Osmolarity**	*Flask*	*External mequiv./l*		*Transport - mequiv./5 mmoles Hgb/4 h*					*Active transport ratio*
			Na	*K*	*Na loss*			*K gain*		
					Net	*Passive*	*Active*	*Net*	*Passive*	*Active*	*K/Na*
1	323	A	143	20	18.2	1.8	20.0	13.5	−0.4	13.9	0.70
		B	73	90	20.4	+1.8	18.6	18.0	−3.0	15.0	0.81
		C	3	160	29.0	+5.3	23.7	21.8	−6.4	15.4	0.65
		D	3	160	7.8			8.6			
2	335	A	147	20	21.5	3.2	24.9	14.3	+2.7	17.0	0.68
		B	77	90	26.4	+0.6	25.8	18.7	−1.3	17.4	0.68
		C	7	160	33.4	−4.5	28.9	23.7	−5.2	18.5	0.64
		D	7	160	5.6			6.9			
3	236	A	100	20	29.1	−2.7	31.8	19.2	+0.9	20.1	0.63
		B	50	70	30.2	+0.8	29.4	24.2	−2.1	22.1	0.75
		C	0	120	38.1	+4.1	34.0	25.9	−5.4	20.5	0.60
		D	0	120	5.2			5.7			
4	436	A	200	20	24.5	−4.2	28.7	15.1	+0.7	15.8	0.55
		B	100	120	30.1	−1.6	28.5	21.0	−3.3	17.7	0.62
		C	0	220	36.7	+7.4	29.3	25.4	7.3	18.1	0.62
		D	0	220	8.5			7.8			

* Osmolarity is molarity multiplied by the number of osmotically active particles which a molecule forms in solution.

Mean 0.66
S.E. ± 0.02

one experiment to another or from one flask to another was of borderline significance ($P = 0.08$) and was not consistently related to the experimental variables.

The stability of the ratio to changes in the pumping rate was tested by abruptly stopping transport with strophanthin (5 mg/l) or arsenate (23 mmoles/l) and by accelerating transport by the addition of adenosine (4 mmoles/l) to cells previously deprived of substrate. No significant alteration of the ratio from the normal value appeared. The ratio was also preserved at pH 6.5 and at pH 7.9.

No significant evidence of linkage at 2° was observed. Fresh cells were stored at 2° in a choline chloride medium containing enough sodium to be in equilibrium with the sodium inside the cell. Potassium left the cells rapidly and they shrank but the sodium content was unaffected. On replacing the external choline with potassium, potassium entered the cells rapidly, the volume increased, and the sodium content remained unchanged. When the parts played by sodium and potassium were exchanged, the results were similar.

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3. *Ammonium*

The addition of ammonium to sodium-filled cells induced maximal active sodium transport in the absence of external potassium. The effect was half-maximal at about 16 mequiv./l of ammonium. This is seven times greater than the corresponding concentration of potassium (2.2 mequiv./l). The results are shown in Fig. 7.

To test for competition between ammonium and potassium, external potassium was adjusted to 10 mequiv./l in order to almost saturate the transport system. The addition of increasing amounts of ammonium now produced only a small increase in sodium transport. It also produced an obvious depression of potassium transport. The results are shown in Fig. 8. These results are consistent with the view that ammonium and potassium compete both in the activation of outward sodium transport and for inward transport.

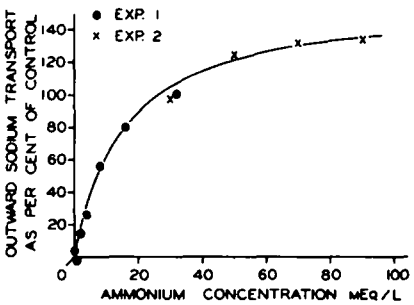

Fig. 7. The effect of ammonium on active sodium transport in the absence of external potassium. The cells were stored at 2° for 13 and 10 days in experiments 1 and 2 respectively. In both experiments they contained initially Na = 105 and K = 17 mequiv./5 mmoles Hgb. The incubation medium contained 160 mequiv./l of sodium and no potassium. Ammonium chloride was added as the solid. To compare the two experiments the rates are related to those in control flasks to which 20 mequiv./l of solid potassium chloride and no ammonium chloride was added. The control rates were 6.4 and 4.6 mequiv./5 mmoles Hgb/h in experiments 1 and 2 respectively. The average of the small leaks at 0 and 1 mequiv./l of ammonium has been added to all the values as a partial correction for passive transport. The smooth curve is drawn from the best-fitting Michaelis-Menten equation where the maximal rate is 160% and the concentration of ammonium at half-maximal transport is 16 mequiv./l. There was a little hemolysis of the cells which had large amounts of ammonium during the wash in cold choline chloride. pH 7.6 to 7.1 ('56.8.03 and .07).

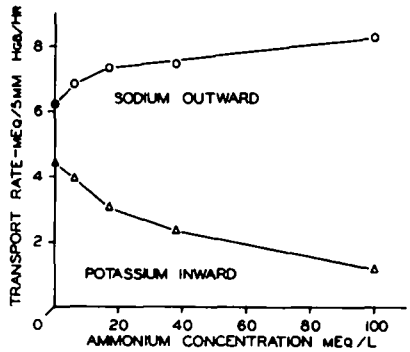

Fig. 8. The competition of ammonium with potassium in the active transport of sodium and potassium. The cells were stored in a sodium medium at 2° for 17 days. At the beginning of incubation at 37° the sodium and potassium contents of the cells were 118 and 13 mequiv./5 mmoles Hgb respectively. The medium contained 150 mequiv./l of sodium and 10 mequiv./l of potassium. Ammonium chloride was added as the solid. There was a little hemolysis of the cells which had large amount of ammonium during the wash in cold choline chloride. pH 7.3 to 7.0 ('56.8.06).

The proposition that ammonium substitutes directly for potassium in an inward transport system which is stoichiometrically linked to an outward sodium transport system can be tested quantitatively for its consistency with the results of previous experiments. The active transport of ammonium (NH_4^+) itself cannot be measured, since ammonia (NH_3) passes through the membrane rapidly, as JACOBS AND PARPART[14] and JACOBS AND STEWART[15] have shown. However, one can start with the assumption that the sum of ammonium transport plus potassium transport is equal to 2/3 of

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sodium transport. On this basis ammonium transport can be calculated by difference. In a one-for-one competition for an inward transport binding site, the ratio of the transport rates would be equal to the ratio of the concentrations multiplied by the reciprocal of the ratio of the dissociation constants. The dissociation constant is the concentration of an ion at which it produces half-maximal transport when acting alone. The ratio of the ammonium dissociation constant to the potassium dissociation constant was calculated on this basis for the four concentrations of ammonium shown and a mean value of 3 was obtained. The corresponding ratio of the dissociation constants previously determined from the independent sodium activation experiments was 7. This degree of correspondence between the ratios is not very impressive at first sight, but the values are of the same order of magnitude. In view of the fact that precise passive transport corrections were not made in any of these experiments, this degree of correspondence is perhaps as close as could be expected.

Ammonium (30 mequiv./l) did not activate potassium transport in low sodium cells and therefore presumably cannot substitute for sodium.

DISCUSSION

The results of others

The data in this paper support the linkage hypothesis of HARRIS[6], except with respect to the ratio of potassium to sodium transport. Using tracer fluxes taken from the literature, HARRIS estimated the ratio as 1 to 2 in normal cells in plasma. TOSTESON[1] in a more recent review of the data has estimated the active potassium influx at 1.9 mmoles/l cells/h and the active sodium outflux at 2.8 mmoles/l cells/h. The ratio of these is 2 to 3, in good agreement with the data in this paper.

MAIZELS[5] agreed with HARRIS that there is a linkage between the transport systems but thought that it is not stoichiometric. In one experiment in particular, he observed a greater influx of potassium in a high potassium medium than he calculated should occur if the transports were tightly linked. He argued that the potassium-sodium ratio must be higher under these circumstances. In his calculations he assumed that the exchange rate of tracer potassium between normal cells and plasma is 1.6 mequiv./l of cells/h. If this is replaced by 2.0 mequiv./l of cells/h taken from TOSTESON[1], much of the discrepancy between the results predicted by the theory of stoichiometric linkage and his experiment disappears. Furthermore, in short-term experiments on horse red cells SHAW[16] has shown that potassium influx can be expressed as the sum of two terms. If the linear term is interpreted as passive influx and the nonlinear term as active influx, the results indicate that there is no change of active potassium transport produced directly by high (> 20 mequiv./l) external potassium concentrations. The results of GLYNN[7] indicate that human and horse cells are similar in this respect.

SOLOMON[17] has argued in favor of the complete independence of the sodium and potassium transport systems. He observed influxes of labelled potassium and sodium in fresh cells in an approximately steady state in plasma *in vitro*. He observed that rubidium but not sodium or lithium competed with potassium for influx, and that lithium but not potassium or rubidium depressed sodium influx. He tentatively assumed that the influx and outflux of each ion are linked by the chemical conversion of influx carrier molecules into outflux carrier molecules. On this basis he felt that

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potassium influx and sodium outflux should be independent. Recently he has dropped the hypothesis that potassium efflux is active[18]. In the light of the evidence available now it appears more likely that influx of sodium and outflux of potassium in the normal cell are passive and independent processes, whereas influx of potassium and outflux of sodium are mostly active and numerically related processes.

The kinetics of active transport

The discrepancy in the pumping rates for sodium and potassium would have a considerable effect on the membrane potential due to the net movement of positive charge if it were not for the fact that the erythrocyte membrane is freely permeable to small anions such as chloride and bicarbonate. HARRIS AND MAIZELS[4] have shown that the membrane potential, as measured by the chloride concentration ratio, is unaffected by the presence or absence of active transport, at least over a period of hours.

The observation that the rate-limiting effect of low external potassium concentrations is half-maximal at 2.2 mequiv./l is in good agreement with the figure of 2.1 mequiv./l of STREETEN AND SOLOMON[19], of 1.8 to 2.5 obtained by GLYNN[7], and of 2.0 to 3.2 mequiv./l obtained by SHAW[16] with horse erythrocytes. It does not support the earlier assumption of HARRIS[6] that external potassium concentrations above 2 mequiv./l saturate the active transport system.

The data presented in Fig. 6 showing the influence of internal sodium concentration on the transport rate are consistent with those of HARRIS[6], who presented evidence that sodium transport is proportional to internal sodium concentration below 40 mequiv./l of cells. The constancy of the transport rate at high internal sodium concentrations indicates that under these conditions internal sodium concentration is no longer rate-limiting.

Biochemical implications

Since stoichiometric relationships between reactants and products are a characteristic of simple chemical reactions, the demonstration that the active transports of sodium and potassium across the human erythrocyte membrane are numerically related gives support to the view, already held by many, that active transport proceeds by way of a chemical combination of the cations with specific binding sites. This demonstration further suggests that a chemical group must pass from the carrier of one cation to the carrier of the other at some stage in the transport processes in order to keep the two systems in exact step with each other. Such a transfer could take place at either or both surfaces of the membrane and could be a transfer from either the potassium carrier to the sodium carrier or *vice versa*.

Physiological implications

HARRIS AND MAIZELS[4] 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. These difficulties could be avoided if the passive permeability of the cell membrane to sodium became greater at the same time that the potassium active transport system disappeared. Under these circumstances the cell would resemble a dog erythrocyte, which actively transports little or no potassium and has a relatively high sodium permeability[20,21].

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Since a two-way linked active transport system is more complicated than a one-way system and since both types apparently exist in nature, it might be asked whether the more complicated two-way system has any conceivable advantage. With an ideal model it can be calculated that for the same cell volume and impermeable particle content a cell with a two-way linked active transport system is better able to stabilize its volume against variations in passive membrane permeability than is a cell with a one-way system. The detailed argument is given in an appendix.

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SUMMARY

1. The net transports of potassium and sodium across the human erythrocyte membrane were observed at 37° in cells prepared by cold storage and fortified with nucleoside. Passive transport was minimized by adjustment of sodium and potassium concentrations in the medium. Active transport was distinguished from passive transport by the use of strophanthin, which stops active transport specifically.

2. The active transport of potassium inward and sodium outward occurred only at a ratio which was constant over a wide range of rates and independent of extracellular and intracellular sodium and potassium concentrations. Two atoms of potassium were transported inward for every three atoms of sodium that were transported outward.

3. Ammonium appeared to substitute directly for potassium and required a concentration 3 to 7 times greater than potassium to produce a comparable effect.

4. These findings indicate that active potassium and sodium transport across the human erythrocyte membrane are parts of a single tightly-linked system.

APPENDIX

A theoretical demonstration of the physiological advantage of linkage in active transport. In theory a linked active transport system can stabilize the volume of a cell more effectively than can a system transporting actively in only one direction. This point can be explained most clearly with a model.

Consider a cell as an aqueous phase, 1, containing two solutes, H and C. The cell is surrounded by a freely extensible water-permeable membrane. Of the solutes only C passes through the membrane; H does not. External to the membrane is a second aqueous phase, 0, containing only C at a constant concentration $[C_0]$. The square brackets indicate concentration and the subscript denotes the phase. A bar, $\bar{}$, indicates the amount of a variable associated with the cell so that the volume of the cell is $\bar{V}$. $\bar{V} = \bar{H}/[H]$. At osmotic equilibrium $[H] + [C_1] = [C_0]$ so that $\bar{V} = \bar{H}/([C_0] - [C_1])$. Now the net rate at which C passes through the membrane is the difference between the rate at which it leaks in, L, and the rate at which it is pumped out, P. $L = k([C_0] - [C_1])$ where k is a leaking coefficient. $P = p[C_1]$ where p is a pumping coefficient. In a steady state $L = P$ and

$$\bar{V} = \frac{\bar{H}}{[C_0]}(1 + k/p) \tag{1}$$

That is, the more permeable the membrane and the less sensitive the pumping rate to $[C_1]$, the larger will be the cell volume.

The stability of the volume of this cell to changes in the permeability of the membrane can be expressed as the derivative $dk/d\bar{V}$ or better as $S = (dk/k)/(d\bar{V}/\bar{V})$. The result of the calculation is:

$$S = 1 + p/k \tag{2}$$

That is, the greater the ratio of pumping to leaking coefficients, the more stable the cell volume is to

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changes in the permeability of its membrane. It is also true that the more stable cell is smaller and has a higher impermeable particle concentration.

Compare this cell with one in which C is divided into two subspecies A and B. Let the leaks now be $L_A = k([A_0] - [A_1])$ and $L_B = k([B_0] - [B_1])$. Let the active transport (outward) of A be $P_A = p[A_1]$. Let the active transport (inward) of B be linked to that of A by a fixed ratio, $r = P_B/P_A$. Note that changes in $[B_1]$ are regarded as having no effect on p. $[A_0]$ and $[B_0]$ do not change in any case. Then $P_B = -rp[A_1]$. At a steady state $L_A = P_A$ and $L_B = P_B$. The result is

$$\overline{V} = \frac{\overline{H}}{[A_0](1 - r)}(1 + k/p) \tag{3}$$

The value of the stability, S, derived from this equation is the same as (2) above.

In comparing the stability of these two systems it is appropriate to make $\overline{V}$ and $\overline{H}$ the same in both. $[A_0]$ can also be made equal to $[C_0]$. Denote k/p by n and the systems by the subscripts u and w for the single and linked systems respectively. In this case from equations (1) and (3)

$$(1 + n_u) = \frac{(1 + n_w)}{1 - r}.$$

Solving for n_u

$$n_u = \frac{n_w + r}{1 - r}.$$

By inspection, as long as $0 < r < 1$, n_u must be greater than n_w. Since $S_u = 1 + 1/n_u$ and $S_w = 1 + 1/n_w$, it follows that under these conditions $S_w > S_u$. This result shows that in theory under equivalent circumstances a cell whose volume is stabilized by an active transport system can be more stable to variations in the leakage of the membrane if the transport system is a linked one than if it is a simple one.

REFERENCES

1 D. C. TOSTESON, *Electrolytes in Biological Systems*, American Physiological Society, Washington, 1955, p. 123.
2 F. FLYNN AND M. MAIZELS, *J. Physiol.*, 110 (1949) 301.
3 E. J. HARRIS AND M. MAIZELS, *J. Physiol.*, 113 (1951) 506.
4 E. J. HARRIS AND M. MAIZELS, *J. Physiol.*, 118 (1952) 40.
5 M. MAIZELS, *Symposia Soc. Exptl. Biol.*, 7 (1954) 202.
6 E. J. HARRIS, *Symposia Soc. Exptl. Biol.*, 7 (1954) 228.
7 I. M. GLYNN, *J. Physiol.*, 134 (1956) 278.
8 R. L. POST, *Federation Proc.*, 15 (1956) 146.
9 R. L. POST, *Am. J. Physiol.*, 187 (1956) 622.
10 H. J. SCHATZMANN, *Helv. Physiol. Acta*, 11 (1953) 346.
11 D. M. DONOHUE, C. A. FINCH AND B. W. GABRIO, *J. Clin. Invest.*, 35 (1956) 562.
12 W. H. CROSBY, J. I. MUNN AND F. W. FURTH, *U.S. Armed Forces Med. J.*, 5 (1954) 693.
13 D. L. DRABKIN AND J. H. AUSTIN, *J. Biol. Chem.*, 112 (1935) 51.
14 M. H. JACOBS AND A. K. PARPART, *J. Cellular Comp. Physiol.*, 11 (1938) 175.
15 M. H. JACOBS AND D. R. STEWART, *J. Gen. Physiol.*, 25 (1942) 539.
16 T. I. SHAW, *J. Physiol.*, 129 (1955) 464.
17 A. K. SOLOMON, *J. Gen. Physiol.*, 36 (1952) 57.
18 A. K. SOLOMON, T. J. GILL, III, AND G. L. GOLD, *J. Gen. Physiol.*, 40 (1956) 327.
19 D. H. P. STREETEN AND A. K. SOLOMON, *J. Gen. Physiol.*, 37 (1954) 643.
20 C. W. SHEPPARD, W. R. MARTIN AND G. BEYL, *J. Gen. Physiol.*, 34 (1951) 411.
21 H. S. FRAZIER, A. SICULAR AND A. K. SOLOMON, *J. Gen. Physiol.*, 37 (1954) 631.

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