STUDIES ON THE SODIUM AND POTASSIUM TRANSPORT IN RABBIT POLYMORPHONUCLEAR LEUKOCYTES

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ABSTRACT

Rabbit polymorphonuclear leukocytes obtained from peritoneal exudates, incubated at 37°C. following exposure to 4°C., actively reaccumulate potassium while little or no net extrusion of sodium takes place. Preventing the utilization of oxidative metabolism with potassium cyanide, 2,4-dinitrophenol, or a nitrogen atmosphere does not inhibit the recovery process. Inhibitors blocking anaerobic glycolysis (sodium iodoacetate and sodium fluoride in low concentrations) completely abolish the capacity to reaccumulate potassium and cause a further dissipation of the sodium and potassium gradients. Water movements have been shown to be secondary to cation shifts. It is postulated that separate transport mechanisms exist for sodium and potassium and that the process of potassium reaccumulation relies on anaerobic glycolysis as a source of energy.

INTRODUCTION

Investigations dealing with the metabolic aspects of electrolyte transport show that, depending on the tissue, the energy required for maintenance or recovery of electrolyte gradients is provided by mechanisms which may vary from completely aerobic to entirely anaerobic systems.

The studies described in this communication were performed on rabbit polymorphonuclear leukocytes, cells known to possess both aerobic and anaerobic metabolic pathways. The results demonstrate that this cell system does not require oxygen for maintenance and restoration of the potassium concentration gradient. It will also be shown that the active transport of potassium in these granulocytes occurs independently of sodium movements.

EXPERIMENTAL METHODS

Rabbit polymorphonuclear white blood cells obtained from peritoneal exudates were used in all experiments. The experiments were designed in the following way:

1. Active metabolism was suppressed by exposing the cells to cold for 18 to 24 hours, allowing sodium to enter and potassium to leave the cells.
2. Restoration of sodium and potassium gradients was then studied by incubating the suspension at 37°C.
3. Metabolic inhibitors were added to the suspension prior to incubation to show

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the dependence of the recovery process on specific metabolic pathways. 300 ml. of an isotonic solution containing sodium, potassium, and chloride in physiologic concentrations and 1 mg./ml. of glycogen was injected intraperitoneally in adult New Zealand Red rabbits of either sex. From 5 to 10 hours later the exudates were harvested by aspiration (without sacrificing the animal) into siliconized glass vessels containing 5 mg. of powdered sodium heparin. Exudates contaminated with blood were discarded. White blood cell and differential counts were done at each experiment. The composition of the cell population did not change when the time interval between injection of the challenging fluid and collection was increased from 5 to 10 hours. After one or two exudates had been obtained from a given animal as described, good cell yields occurred when only saline-potassium solution (without glycogen) was injected. Leukocytes from a "glycogen" and a "non-glycogen" exudate were not detectably different in any of the aspects studied. Depending on the cell concentration of the unmodified exudate, 10, 15, or 20 ml. aliquots were pipetted into tared siliconized glass tubes. At time zero (T_0), *i.e.*, immediately after distribution, two of these tubes were centrifuged for 20 minutes at 1700 *g*, care being taken to increase the centrifugal field to 400 *g* within 30 seconds in order to reduce cation exchange with the medium during centrifugation (1). Remaining tubes were attached to a shaking-plank and placed at 4°C. for 18 to 24 hours.

After this period of time, duplicate tubes were removed from the cold and either centrifuged immediately to determine the effect of low temperature on sodium and potassium content after various periods of exposure, or incubated in a water bath at 37°C. under continuous agitation to determine the capacity of the cells to accumulate potassium and extrude sodium. When the effect of metabolic inhibitors was investigated, the inhibitory agent was added immediately prior to warming.

Following centrifugation and removal of a sample of the supernatant for determination of pH, glucose and sodium and potassium concentration, the tubes were inverted and allowed to drain for 5 minutes. Fluid remaining on the sides of the tubes was removed by suction. The tubes were wiped with a clean towel, stoppered, and allowed to equilibrate to 20°C. before weighing for determination of "wet weight." "Dry weight" was obtained by drying to constant weight in an oven at 150°C. for 18 hours. The dried cell-button was digested in concentrated nitric acid, quantitatively transferred to a volumetric flask containing the appropriate amount of lithium chloride, and prepared for sodium and potassium determination by flame photometry (Perkin-Elmer, model 52A) using lithium as an internal standard. All sodium and potassium values were expressed as milliequivalents per kg. of dried cells (*content*) and per liter of cell water (*concentration*).

During the course of the experiment, cell viability was followed by staining with a drop of trypan blue (2) for 5 to 10 minutes and counting the number of cells with stained nuclei. In certain experiments, phagocytic activity and motility were appraised by direct and phase contrast microscopy.

Metabolic Inhibitors:

The following metabolic inhibitors were added to the medium to provide final concentrations in the indicated ranges: sodium iodoacetate (NaIAc) 0.0001 to 0.015 M,[1]

[1] Usually the experiment was carried out with a NaIAc concentration of 0.0001 M.

sodium fluoride (NaF) 0.01 to 0.025 M, potassium cyanide (KCN) 0.0001 to 0.0025 M, and 2,4-dinitrophenol (DNP) 0.0001 to 0.0025 M. The effect of these inhibitors on respiration was studied employing standard manometric methods (3).

A nitrogen atmosphere was created by replacing the air over the cell suspension with a stream of nitrogen and subsequent sealing of the tubes.

Extracellular Fluid:

In two experiments the amount of trapped extracellular fluid in the cell-button after centrifugation was estimated with I^{131}-labelled albumin (Abbott's Laboratories, New York).

An aliquot of the radio-iodinated albumin was first mixed with carrier, 50 mg. of bovine albumin (Armour Laboratories) in 10 ml. of isotonic saline. This mixture was dialyzed in a cellophane bag for 2 hours against 2 liters of water under constant agitation with a magnetic stirrer to remove free I^{131} and I^{131} attached to small molecules (4).

0.8 ml. of the freshly dialyzed solution was added to each 10 ml. sample of white blood cell suspension. After thorough mixing, the tubes were centrifuged immediately and an aliquot of the cell-free supernate was transferred to a counting tube. The cell-button was weighed, dried, reweighed, digested in nitric acid, and quantitatively transferred to a counting tube. The radioactivity of supernate and cells was determined in each sample in a well-type scintillation counter. Trapped extracellular fluid was calculated as the ratio of the radioactivity of the cell-button to that of the supernate:

$$\text{Mg. extracellular fluid} = \frac{\text{Counts per minute/whole cell-button}}{\text{Counts per minute/gm. of supernate}} \times 1000$$

RESULTS

1. Composition of Ascitic Fluid:

Unmodified exudate was employed since it provided a physiologic medium with effective buffer systems. Furthermore, manipulation of the cells was limited to a single transfer, and centrifugation was avoided until final separation of cells and fluid. These advantages were considered to outweigh the disadvantages of possible variations in composition of the fluid phase of the raw exudate.

Concentrations of sodium and potassium in the supernate of the exudate were within the normal range for extracellular fluid (sodium 145 ± 4.9 m. eq./liter; potassium 4.1 ± 0.6 m. eq./liter in 10 exudates). Changes in intracellular cation content did not significantly influence the concentration in the medium, since cell volume did not exceed 2 per cent of the volume of the supernate.

The specific gravity ranged from 1.007 to 1.012 in 8 exudates.

The pH of the supernate of the exudates, immediately following centrifugation of the first sample, ranged from 7.60 to 8.15. This range was maintained during the course of all experiments with the following exception. During measurements of oxygen uptake in a Warburg apparatus the reaction of the medium gradually dropped to pH 6.2 in 4 to 6 hours. This drop occurred both in Krebs-Ringer phosphate medium and in unmodified but concentrated exudate, and was associated with a gradual decrease in oxygen uptake. The fall in pH of the

medium in the Warburg vessels probably reflects the higher cell concentration, resulting in greater lactic acid production.

The glucose level in 14 exudates averaged 87.5 mg., per cent, with a range from 50 to 162 mg. per cent.

2. Cell Population:

White blood cell counts averaged 10×10^6/ml., with a range from 3 to 15×10^6/ml.; virtually all of the cells were polymorphonuclear leukocytes. Variations in cell distribution due to clumping made the cell count unsuitable as a standard of reference, and instead the dry weight of the cells was selected because

TABLE I

Cell Mass of Polymorphonuclear Leukocytes, Expressed as Cell Count, P and N_2 Content, in Relation to Dry Weight*

Type of exudate	Dry weight	P	N_2
		μg./mg. dry wt.	*Per cent of dry wt.*
Caseinate, Glycogen (Mixed cells)‡	1 mg. ~ 7×10^6 cells	13.0	
	1 mg. ~ 5.4×10^6 cells	12.5	
Glycogen (>95 per cent PMN)§	1 mg. ~ 5.4×10^6 cells (average value of 5 experiments)	12.9, 11.7, 11.8 → 12.1 (Average)	Triplicate sample: 14.71 per cent, 14.04 per cent, 13.86 per cent

* As the samples were derived from different exudates, we may deduce from the N_2 data, that the use of the unmodified exudate does not give rise to greatly varying amounts of trapped protein (fibrin), which could be a factor of error in the electrolyte content determinations on the basis of weight.

‡ Stähelin, H., Suter, E., and Karnovsky, M. L.: *J. Exp. Med.* **107**, 121, 1956 (5).

§ Observed.

of the homogeneity of the cell population. Cell counts done when clumping was minimal permitted comparison between various parameters used. Table I shows that the reported values are in the same range as that found by others (5).

Viability.—At T_0 not more than 2 to 5 per cent of the cells showed nuclear staining with trypan blue. After 24 hours at 4°C., 10 to 20 per cent, and after incubation at 37°C. a maximum of 20 to 25 per cent of the cells took up the dye. Poisoning with NaIAc usually caused staining of an additional 5 to 10 per cent. After centrifugation and teasing the cells apart on a glass slide, 30 to 50 per cent of the cells were stained. In disagreement with Tullis' observations (6), it was found that cells which had taken up trypan blue not infrequently displayed active ameboid motion or even phagocytosis, suggesting that dye

uptake is a more sensitive measure of physiologic integrity than motility and/or phagocytosis.

3. Electrolyte Content of Rabbit Polymorphonuclear Leukocytes:

Fresh polymorphonuclear leukocytes contained (T_0) 98 ± 21 m. eq. of sodium and 95 ± 18 m. eq. of potassium per liter of cell water and 414 ± 78 m. eq. of sodium and 400 ± 67 m. eq. of potassium per kilo dry weight (Table II).

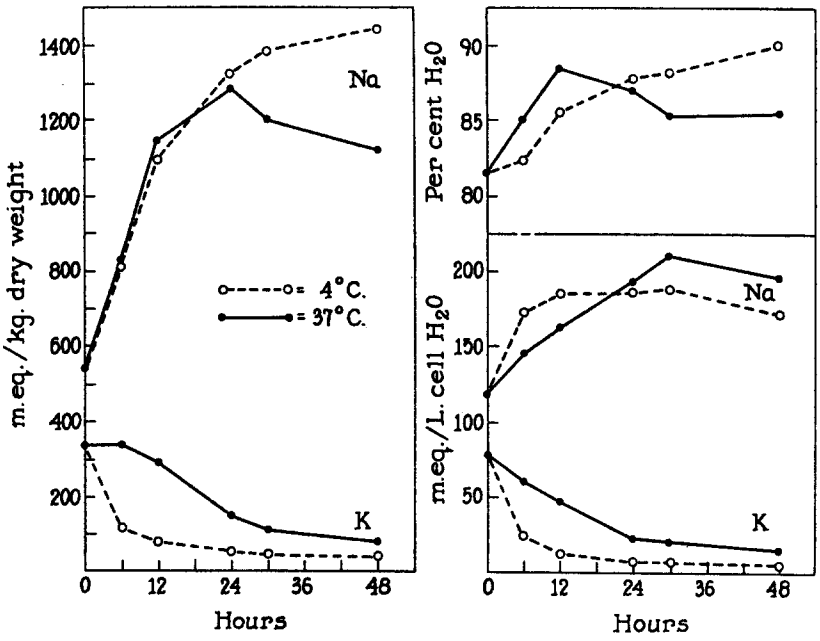

FIG. 1. Changes in Na and K content and concentration of rabbit polymorpho nuclear leukocytes during incubation at 37°C. and exposure to 4°C. for 48 hours.

Fig. 1 demonstrates the changes in sodium and potassium concentration and content during incubation at 37°C. and exposure to 4°C. for 48 hours. It appears that the changes in sodium and potassium concentration were closely reciprocal, both at 37°C. and at 4°C., but that changes in sodium and potassium content were not.

At 37°C., the potassium content was maintained for 6 hours, while sodium and water rapidly entered the cell-button (Fig. 3, first and last column). After 6 hours the potassium content began to fall and continued to do so over the next 42 hours. The sodium content reached a maximum after 24 hours. The increase in sodium content of the cell-button exceeded by far the decrease in

potassium content at all times. After 24 hours the sodium and water content of the cell-buttons diminished, while the sodium concentration increased. These changes may be reflections of the rise in sodium concentration in the medium from 145 to 170 m. eq./liter, due to evaporation of water during the prolonged incubation at 37°C. Also of possible importance was loss of cell substance under these conditions; after 24 hours at 37°C., 30 to 35 per cent and after 30 hours, 70 to 80 per cent of the cell nuclei were stained with trypan blue and disintegrating cells were seen on the slide.

At 4°C. the rise in sodium and drop in potassium concentration of the cell-button were again closely reciprocal. These changes in concentration occurred more rapidly at 4°C. than at 37°C., probably because of the slower increase in water content at the lower temperature.

It is interesting that the increase in sodium content at 4°C. was identical with that observed at 37°C. The potassium content, however, decreased rapidly, but again did not match the increase in sodium content. The changes in concentration as well as content of both cations were nearly maximal after 24 hours at 4°C.

4. Recovery after Exposure to 4°C.:

When incubated at 37°C., following exposure to cold, rabbit polymorphonuclear leukocytes reaccumulated potassium. The extent of restoration of the potassium gradient was not noticeably altered by varying cold exposure between 18 and 24 hours.

Fig. 2 contains the pooled data of two representative experiments. The potassium values are indicated by a single line since they were identical. Potassium reaccumulation was fast, nearly 50 per cent occurring during the first 15 minutes and most of recovery within the 1st hour of incubation. The sodium values are indicated by two lines, each point on the lines representing the averaged duplicates for the two experiments. The variations in the sodium movements are in marked contrast to the consistency observed in the reaccumulation of potassium.

The lack of reciprocity between sodium and potassium movements, seen during both cooling at 4°C. and prolonged incubation at 37°C., was even more striking during recovery from cold exposure, as is seen in the pooled data of all experiments. (See Table II, 3 top lines, and Fig. 3 *a*, first 3 columns). Changes in the sodium content of the cell-button during the recovery phase were small and inconstant in comparison to those for potassium. The small drop in sodium concentration was, however, statistically significant, and may well have been due to further entry of water during incubation. This can be explained by a rise in total cation content due to the reaccumulation of potassium ions unmatched by an equivalent extrusion of sodium.

5. The Effect of Metabolic Inhibitors:

The effect of metabolic inhibitors was studied during the first 60 minutes of recovery at 37°C. following prolonged cold exposure, because recovery is close to maximal at the end of this time interval. Two types of inhibitors were used, one preventing the utilization of oxidative metabolism and one inhibiting enzymes necessary for anaerobic glycolysis.

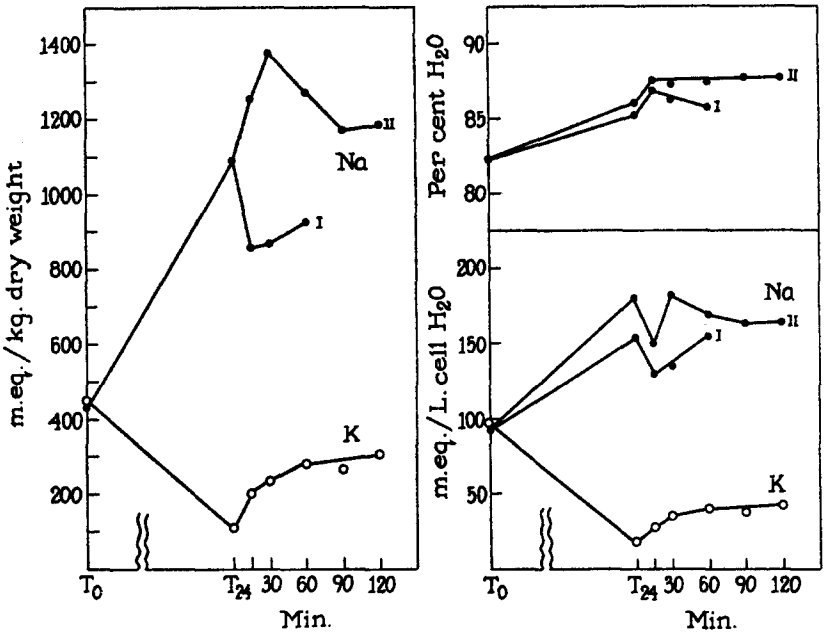

FIG. 2. Changes in Na and K content and concentration of rabbit polymorphonuclear leukocytes during incubation at 37°C. following exposure to 4°C.

Manometric observations showed that KCN in a concentration of 0.015 M effectively blocked respiration for periods up to 6 hours. In concentrations of 0.0015 and 0.00015 M, KCN inhibited oxygen uptake for about 75 minutes, after which respiration resumed. NaIAc in a concentration of 0.0001 M did not affect oxygen uptake during the 1st hour following the addition of the inhibitor; thereafter, a depression of oxygen uptake was sometimes observed. NaF in a concentration of 0.025 M stimulated oxygen consumption for about 1 hour. This was followed by a gradual decrease in respiration. DNP in a concentration of 0.0025 M caused an increased oxygen uptake, as expected.

The effects of these inhibitors on the restoration of sodium and potassium gradients are summarized in Table II and Fig. 3. It is evident that KCN, DNP,

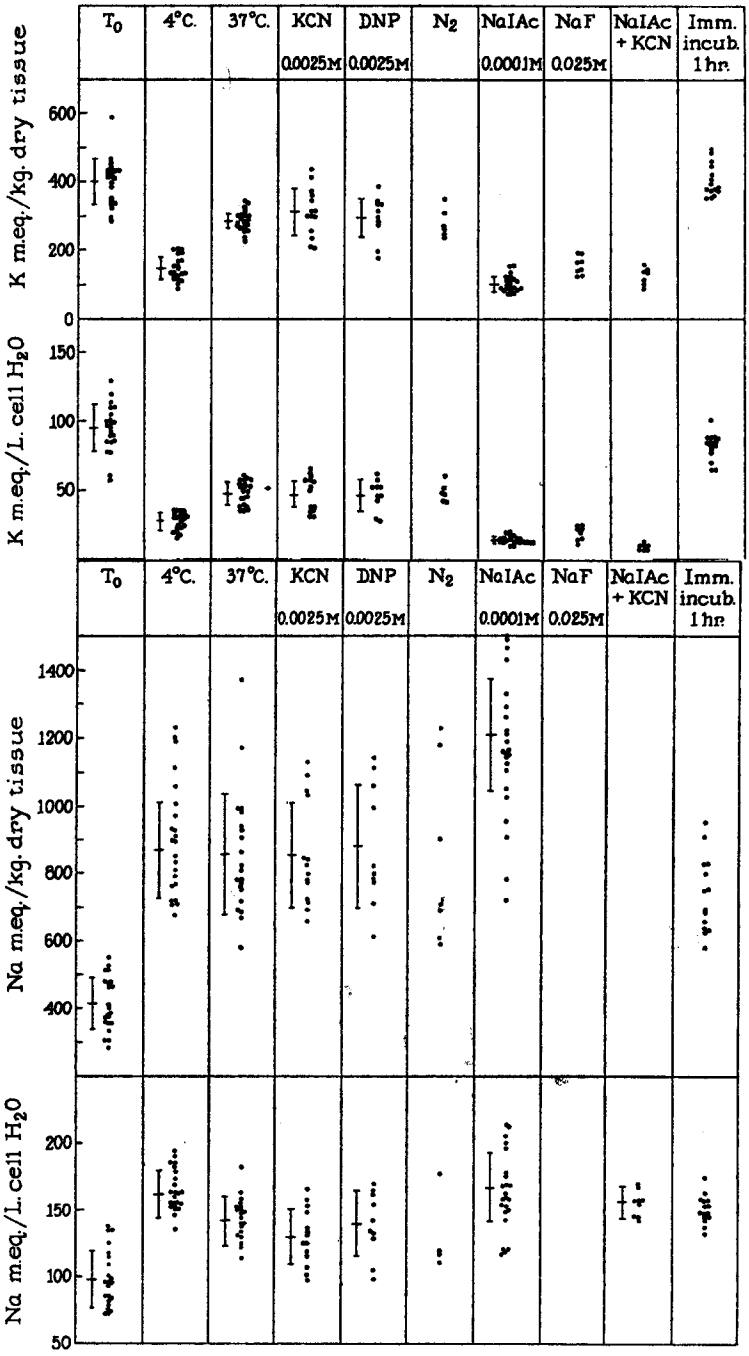

FIG. 3 *a*. Changes in Na and K content and concentration of rabbit polymorphonuclear leukocytes during various experimental conditions.

and a N_2 atmosphere did not prevent potassium reaccumulation or significantly influence the sodium content or concentration.

In two experiments it was observed that KCN and DNP not only failed to suppress recovery, but actually exerted a stimulating effect on potassium reaccumulation, without clearly affecting sodium extrusion. The results of one of these experiments appear in Table III.

In contrast, inhibition of anaerobic glycolysis by NaIAc and NaF had a striking effect on potassium accumulation during recovery from cold exposure. NaIAc in a concentration of 0.0001 M completely blocked recovery and caused a further dissipation of the potassium gradient, while more sodium and water

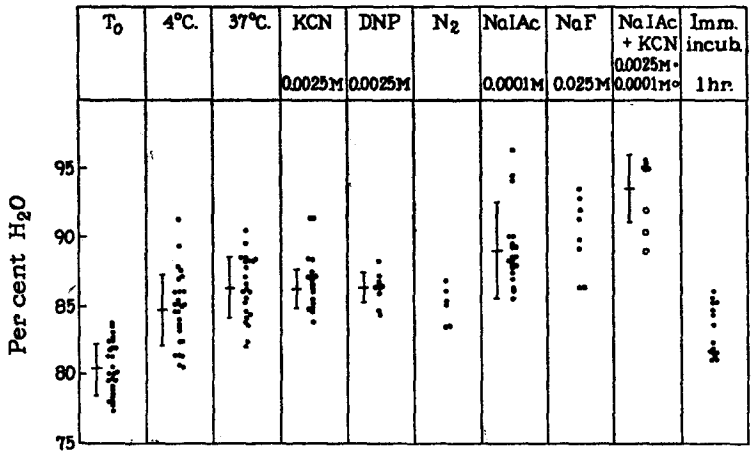

FIG. 3 *b*. Change in water content of rabbit polymorphonuclear leukocytes during various experimental conditions.

entered the cell-button (Table II and Fig. 3). NaF in a concentration of 0.025 M had a similar effect. With a 0.01 M solution of NaF, recovery was partially inhibited.[2]

Glucose consumption was abolished by both NaIAc and NaF; with these agents a rise in glucose level in the medium over the control value was observed repeatedly.

The sodium and water content of the cell-button increased more in the presence of both NaIAc and KCN than in the presence of NaIAc alone. The fall in potassium concentration observed under these conditions can be accounted for by entry of water because the potassium content did not fall (Table II).

[2] Martin (7) has demonstrated that lactate production by mixed human leukocytes is almost completely abolished by 0.0001 M NaIAc and 0.025 M NaF and partially suppressed by 0.01 M NaF.

The degree of inhibition over and above that caused by NaIAc alone was roughly proportional to the concentration of KCN used. The cell-button of a sample treated with both inhibitors appeared gelatinous. Microscopically most of the cells of such buttons were disintegrated and grossly swollen nuclei were

TABLE II*

Summary of the Average Values Showing the Changes in Na and K Content of polymorphonuclear Leukocytes During the Experiment

	1‡ Na/kg. dry weight	2 Na/liter cell H_2O	3 K/kg. dry weight	4 K/liter cell H_2O	5 Na gained/ kg. dry weight	6 K lost /kg. dry weight	7 Excess Na gained over K lost	8 Per cent H_2O	9 H_2O/ kg. dry weight	10 Increase in H_2O over T_0/ kg. dry weight	11§
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m .eq.*	*m. eq.*	*m. eq./ kg. dry weight*		*liters*	*liters*	
T_0	414 ± 78 (22)	98 ± 21.3 (22)	400 ± 67.5 (22)	95 ±.17.7 (22)				80.4 ± 1.9 (26)	4.10		
4°C.	867 ± 142 (21)	162 ± 17.9 (21)	147 ± 35 (21)	28 ± 6.4 (21)	453	253	200	84.7 ± 2.6 (27)	5.54	1.44	216
37°C.	855 ± 181 (21)	142 ± 18.6 (21)	285 ± 20.7 (21)	48 ± 8.7 (21)	441	115	326	86.3 ± 2.3 (21)	6.30	2.20	330
KCN	852 ± 156 (14)	130 ± 20.9 (14)	311 ± 70 (14)	48 ± 9.2 (14)	438	89	349	86.2 ± 1.4 (16)	6.25	2.15	322
DNP	880 ± 182 (10)	140 ± 24.6 (10)	295 ± 57 (10)	47 ± 11.5 (10)	464	105	359	86.3 ± 1.1 (14)	6.30	2.20	330
NaIAc	1207 ± 165 (21)	167 ± 26.6 (21)	104 ± 24.5 (21)	14 ± 2.5 (21)	793	296	497	89.0 ± 3.6 (21)	8.09	3.99	598
NaIAc + KCN	2595 ± 876 (9)	157 ± 12.4 (9)	148 ± 50 (9)	9 ± 2.4 (9)	2181	252	1929	93.5 ± 2.5 (9)	14.38	10.28	1542

* T_0 = first sample; centrifuged immediately after collection of exudate.
4°C. = after approximately 20 hours rocking at 4°C.
37°C. = incubation at 37°C. for 1 hour, after 20 hours at 4°C.
Inhibitors added prior to incubation.

‡ Columns 1 to 4 and 8 show the average values for Na and K with their standard deviations. The figures between parentheses indicate the number of observations.

§ Column 11 shows the calculated amount of Na, contained by the observed increase in hydration over T_0 (column 10), assuming that this water represents entry of extracellular fluid with a Na concentration of 150 m.eq./liter into the cell-button.

seen, surrounded by granules and nuclear remnants. Obviously most of the cells were not viable.

6. Lack of Reciprocity between Sodium and Potassium Movements:

Lack of reciprocity between sodium and potassium movements was seen under all conditions studied. All data bearing on sodium and potassium shifts are presented in Table II. Columns 1 to 9 are self-explanatory. Column 10 repre-

sents the increase in water content over T_0, expressed as liters of water per kilo of dry tissue. Column 11 shows the calculated sodium content of this amount of water, if assumed to be extracellular fluid with a sodium concentration of 150 m. eq./liter. Despite the close agreement of the values in column 7 and 11, the sequence of the changes in sodium and potassium content shows

TABLE III*

Example of Stimulatory Effect of DNP and KCN on the Reaccumulation of K by Rabbit Polymorphonuclear Leukocytes

(The results shown represent duplicate determinations in a single experiment)

	Na/kg. dry weight	Na/liter cell H_2O	K/kg. dry weight	K/liter cell H_2O	Per cent H_2O
T_0	374	96	434	111	79.6
	461	99	557	98	82.3
4°C.	1110	155	118	19	86.0
	1190	153	122	18	87.1
37°C.	1370	183	291	38	88.2
	1170	155	270	36	87.3
DNP (0.0005 M)	1060	165	338	53	86.5
	1140	154	344	46	88.2
DNP (0.0001 M)	1110	170	338	52	86.7
	995	163	388	63	85.9
KCN (0.0025 M)	1040	157	438	66	86.8
	1130	166	413	61	87.1

* T_0 = first sample; centrifuged immediately after collection of exudate.
4°C. = after approximately 20 hours rocking at 4°C.
37°C. = incubation at 37°C. for 1 hour, after 20 hours at 4°C.
Inhibitors added prior to incubation.

that the increased hydration does not represent a simple entry of extracellular fluid into the cell-button.

The I^{131} albumin space of the packed cell-button was determined to estimate how much of the increase in hydration and sodium content was due to an increase in trapped extracellular fluid. At T_0 this space amounted to 15 per cent of the wet weight. A correction was made for the amount of label which adhered to the walls of the tube following decantation of the supernate. This radioactivity (*ca* 25 per cent of that trapped in the packed cells) was carried over into the counting tube, as acid digestion of the cell-button removed most of the label from the glass.

Table IV compares the increase in water content of the cell-button during the course of the experiment, calculated from the observed weight changes, with the increase in I^{131}-labelled albumin space, calculated from trapped radioactivity (uncorrected for adsorption onto glass since this error is presumably

TABLE IV*

Experiment Showing the Percentage of Trapped Extracellular Fluid Contributing to the Increase in Water Content of the Cell-Button

	1 Wet weight	2 Dry weight of cell-button	3 H_2O	4 H_2O/mg. dry weight	5 Increase in H_2O/mg. dry weight	6‡ Gain in H_2O/sample	7§ Extracellular fluid	8 Extracellular fluid as per cent of wet weight	9 Gain in Extracellular fluid over T_0	10‖ Extracellular fluid as per cent of increase in H_2O content
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*		*mg.*	
T_0	162.1	32.5	129.6	3.98			35.4	22.0		
	164.4	32.6	131.8	4.04			31.6	19.3		
	172.3	33.4	138.9	4.16			33.3	19.3		
	181.0	33.0	148.0	4.48			35.7	19.7		
4°C.	171.5	33.0	138.5	4.20	−0.12	−4.0	40.0	23.3	6.0	
	163.5	28.8	134.7	4.68	+0.36	10.4	35.7	21.8	1.7	16
37°C.	172.0	26.1	145.9	5.59	1.27	33.1	40.0	23	6.0	18
	174.0	28.9	145.1	5.02	0.70	20.2	45.0	26	11.0	54
KCN	182.3	27.5	154.8	5.63	1.31	36.0	50.0	27	16.0	44
	177.1	29.1	148.0	5.08	0.76	22.0	50.0	28	16.0	73
NaIAc	209.5	27.2	182.3	6.70	2.38	65	111.0			
	231.6	25.4	206.2	8.12	3.80	96	71.4	31	37.4	39
NaIAc + KCN	1474.6	37.5	1437.1	38.22	34.72	1302	549		515.0	39
	1510.2	36.7	1473.5	40.15	36.55	1341	526		492.0	37

* T_0 = first sample; centrifuged immediately after collection of exudate.
4°C. = after approximately 20 hours rocking at 4°C.
37°C. = incubation at 37°C. for 1 hour, after 20 hours at 4°C.
Inhibitors added prior to incubation.

‡ The figures in column 6 are obtained by multiplying those in column 2 and 5.

§ Extracellular fluid as determined by the I^{131}-labelled albumin space of the packed cell-button. (Uncorrected for the radioactivity adsorbed onto glass.)

‖ Values of column 9, as percentages of values appearing in column 6.

the same in all samples). It is evident that trapped extracellular fluid can account for only part of the increase in hydration of the cell-button.

DISCUSSION

1. Sodium and Potassium Content of Rabbit Polymorphonuclear Leukocytes:

The relatively high ratio of sodium to potassium in leukocytes of fresh rabbit peritoneal exudates was reported previously by Wilson and Manery (8) and by Hempling (9). There are no direct data showing whether or not this ratio obtains in the leukocytes in the circulating blood or in the peritoneal exudate

before it is drawn. The few available reports on peripheral blood (10–12), do not provide relevant material for comparison.

The observed high intracellular sodium content could be due to either entry of sodium in association with anions or exchange of sodium for potassium during the 60 to 90 minute interval necessary for preparing the T_0 sample. No direct evidence is available to demonstrate or to exclude these possibilities. However, the cells appear to be in a metabolic steady state at room temperature at the time that the first sample was prepared, since no change in sodium and water content occurred during periods of up to 2½ hours, under these conditions. Moreover, the sodium and water content at T_0 bore no parallel relationship, despite a wide range of variation of each. That sodium did not exchange with cell potassium during processing of the first sample was suggested by the fact that the potassium content subsequently remained constant for at least 6 hours both at room temperature and at 37°C. If, indeed, early rapid exchange did occur, a multiple compartment system has to be assumed as appears to be the case in red blood cells (13, 14), kidney tissue (15), and frog muscle (16).

No unidirectional fluxes were measured in this study, but the possibility of multiple cation compartments was explored in experiments in which evidence for cation-binding was sought. Fresh packed leukocytes were disrupted as follows: (1) freezing and thawing, which presumably leaves the large molecules intact; (2) sonic disintegration which causes a varying amount of destruction; (3) dissolution of the cells in concentrated nitric acid, which liberates all intracellular sodium. After separation of the cell debris, the supernates of (1) and (2) had identical sodium and potassium concentrations as the acid digest of the intact cell-button, suggesting that all sodium and potassium are freely exchangeable. Moreover, Hempling (9) found that extraction of cell-buttons with distilled water for sodium and potassium analyses gave the same values as ashing and acid digestion.

2. Restoration of Electrolyte Gradients:

The capacity of rabbit polymorphonuclear white blood cells to reaccumulate potassium is maintained for at least 24 hours. This time interval is longer than reported for any other normal nucleated mammalian cell; most tissues lose the capacity to reaccumulate potassium within a few hours. A complete recovery of sodium and potassium gradients could not be achieved under the conditions employed. It was shown that potassium reaccumulation occurred in a predictable and constant manner, while net sodium extrusion was usually not observed (Table II). If the sodium content did drop during incubation, the amount expelled never matched the quantity of potassium regained.

3. Effect of Inhibitors:

The most remarkable aspect of the agents used in these experiments was the absence of any inhibitory effect on potassium transport by KCN, DNP, and a

N_2 atmosphere. KCN was used in dosages which abolished oxygen uptake and 2,4-DNP in concentrations known to uncouple oxidative phosphorylation. One may thus conclude that oxidative metabolism, although present in these cells, is not required for the maintenance or restoration of the potassium concentration gradient. On the other hand, NaIAc and NaF in concentrations which blocked glycolysis but did not reduce oxygen uptake, not only prevented recovery, but caused a further loss of potassium and gain in sodium and water. No other nucleated mammalian cell system shows a similar dependence on glycolysis.

The additional changes in sodium and potassium content, observed when KCN as well as NaIAc were present in the suspension, were associated with striking morphological alterations to the point of cell disintegration. It may be suggested that during cell disintegration, factors such as increase in intracellular osmotic pressure and in the number of anionic sites, account for entry of medium in bulk as well as sodium in particular. Therefore, the additive effect of KCN is not considered to be an uncovering of a role of oxidative metabolism in cation transport, but rather to be attributable to a general toxic effect and to an elimination of all metabolism, including that subservient to the maintenance of cell structures.

An interesting observation in two experiments was the unmistakably stimulatory effect of KCN and DNP on the reaccumulation of potassium. It is well known that inhibition of respiration often leads to stimulation of glycolysis in cells which possess both oxidative and anaerobic metabolic pathways, such as nucleated red blood cells and mouse ascites tumor cells (17, 1). Previous workers have not observed that this increase in glycolysis was associated with an increase in electrolyte transport.

4. The Lack of Reciprocity between Sodium and Potassium Movements:

It was shown, under the different conditions of these experiments, that the amount of sodium gained in excess of the amount exchanged for potassium, was matched by an isotonic increase in water content of the cell-button. From 20 to 30 per cent of this increment was accounted for by an increase in trapped extra-cellular fluid (Table IV). The remainder, *i.e.*, the intracellular increment, was shown not to represent simple entry of extracellular fluid into the cells, particularly during the recovery phase when potassium reaccumulated while the sodium content did not change. In these experiments the reaccumulation of potassium was clearly not linked to net sodium movements in the opposite direction.

Leaf (18) demonstrated that water fluxes may mask unreciprocal sodium and potassium movements in rat kidney, liver, and brain slices and that expression of the cation content per unit dry weight is needed to make the unequal flow apparent. Swelling has been observed in a variety of tissues under unfavorable

metabolic conditions, such as cold exposure. This entry of water has been associated with an influx of sodium in excess of potassium lost (15, 18), indicating that sodium enters in association with anion as well as in exchange for potassium.

During favorable metabolic conditions, for example during recovery of erythrocytes following cooling, sodium expulsion exceeded potassium reaccumulation with a net cation loss (19). On the other hand, potassium reaccumulation by mouse ascites tumor cells was found by Hempling (20) to be more effective than sodium extrusion; while Maizels *et al.* (1), using the same cell system, concluded that a 1:1 linkage of sodium and potassium transport exists, and that Donnan effects and anion shifts caused apparent inequality of sodium and potassium fluxes. Deyrup (21) using rat renal cortex slices observed extrusion of sodium and water, unaccompanied by potassium reaccumulation, and she emphasized that in the metabolic regulation of tissue water and electrolytes, a simple obligatory relationship between the cation movements need not exist. In a recent paper Cort and Kleinzeller (22) demonstrated inequality of reciprocal sodium and potassium fluxes in dog kidney cortex slices. Their analysis suggested that in this tissue sodium is actively transported whereas potassium follows electrochemical gradients only.

The results of the present study are consistent with the findings reviewed above, in that potassium reaccumulation was not coupled to sodium extrusion. This suggests a different effectiveness of the transport mechanism for sodium and potassium or the existence of two independent mechanisms.

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