POTASSIUM AND SODIUM MOVEMENTS IN THE EHRLICH MOUSE ASCITES TUMOR CELL*

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(Received for publication, July 2, 1957)

ABSTRACT

Studies have been conducted on the movements of sodium and potassium into and out of the Ehrlich ascites tumor cell. Under steady state conditions, at 22°C., in the absence of an exogenous source of glucose, the cell flux for both potassium and sodium averaged 0.8 μM/10^7 cells/hr. or 3.0 pμM/cm.^{2}/sec. The cell can accumulate potassium and extrude sodium against electrochemical gradients for both ions. It is possible under the experimental conditions reported to separate the transport systems for these two ions. Thus, it has been shown that under conditions of low temperature with a diminished metabolism, net fluxes for the two ions are different. Also, following periods of 24 hours at 2°C., an exogenous source of glucose enhances the accumulation of potassium sevenfold while sodium extrusion is uninfluenced by the presence of glucose. Similarly potassium exchange rates are temperature-dependent, with Q_{10} values as high as 5, while exchange rates for sodium are temperature-insensitive, with Q_{10} values of 1.2 to 1.6. Glycolysis has been eliminated as an energy source for the transport processes since these processes go on in the absence of an exogenous source of glucose. It is estimated that a maximum of 0.3 per cent of the energy derived from the total oxidative metabolism of glucose would be required to support independent transport of potassium and sodium.

INTRODUCTION

The Ehrlich ascites tumor cell provides a useful tool for the study of the interrelationships between cell metabolism and electrolyte transfers. The cell is approximately 16 micra in diameter and spherical in shape. It grows and divides rapidly. As little as 0.2 ml. of a cell suspension when injected intraperitoneally into a mouse will yield after 7 days as much as 10 ml. of a suspension containing 2 gm. of cells. In this time, the host mouse will divert its energy to producing 8 to 10 per cent of its body weight in foreign tissue (1).

The tumor cell exhibits glycolysis, both anaerobic and aerobic, with only a minimal Pasteur effect, producing large amounts of lactic acid. The cell also

* This work was supported in part by a grant from the National Cancer Institute, United States Public Health Service.

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has the peculiar property of a reverse Pasteur effect; *i.e.*, the inhibition of respiration by glycolysis when large amounts of glucose are added (2–5). In addition, it shows the strongest activity for the concentrative uptake of amino acids which so far has been observed *in vitro* for a tissue of mammalian origin (6, 7).

Since little work is available with regard to the behavior of the ascites cell toward electrolytes, this communication will serve to develop the basic framework and potentialities of this cell.

Methods

1. Ehrlich Ascites Tumor Cells (4n).—The strain used in these investigations was the tetraploid and was obtained originally from Dr. M. R. Lewis of the Wistar Institute. Dependent upon the needs of the study, the tumor was carried either in Swiss mice or frozen and kept at −50°C. until a time when the line was reestablished by transplanting thawed cells again into Swiss mice.

2. Cell Suspensions.—Cell exudates were obtained by aspiration from the peritoneal cavities of several mice carrying cell populations ranging in ages from 5 to 10 days. The mixed cell suspension was freed of red blood cells by differential centrifugation (4). This usually required two to three washes. The wash and suspending medium was a K–Na Ringer solution.[1] Whether or not glucose was included depended upon the experiment. Glucose, when present, was in concentrations of 100 mg. per cent.

3. Sampling and Analytical Techniques.—Cell counts were made on cell suspensions, usually prior to mixing of a known volume of a concentrated cell suspension with a known volume of the environment being studied. A standard Neubauer-Levy hemacytometer was used and one thousand or more cells were counted.

A known number of cells were packed and dried to constant weight in an oven at 80–90°C. It was found that 3.6 mg. dry weight corresponded to 10^7 cells.

Aliquots of cell suspension were concentrated by mild centrifugation, rinsed quickly (in less than 3 minutes) with 5 per cent dextrose or 10 per cent sucrose solutions, and then packed in small tubes at 2300 *G* for 20 minutes. The extent of packing was comparable to experiments in which the air turbine was used (8).

The packed cells were weighed and then taken up to a final volume of 10 ml. with distilled water. After 30 minutes or longer, the cell debris was centrifuged and analyses were made on the supernatant using the Baird model flame photometer.

Dry weights were obtained by drying to constant weight at 80–90°C. and per cent water was calculated in the usual fashion.

For the determination of hydrogen ion production, the cells were separated from

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[1] 9 gm. NaCl
40 ml. of 0.154 M KCl solution
15 ml. of 0.11 M NaH_2PO_4
85 ml. of 0.11 M Na_2HPO_4
To a liter with distilled water

the suspending medium and a sample of the supernatant was removed. The supernatant was titrated with standard NaOH solution, using phenolphthalein as an indicator. This gave a measure of the millimoles of $H_2PO_4^-$ present. Back titration with standard HCl solution, using methyl red as an indicator, measured the total $HPO_4^=$ present so that subtraction from the total $HPO_4^=$ of the amount of $H_2PO_4^-$ converted to $HPO_4^=$ with NaOH gave a measure of the amount of $HPO_4^=$ in the environment. The amounts of $HPO_4^=$ and $H_2PO_4^-$ present in control samples were compared with amounts in samples obtained from cell suspensions incubated at 37°C. in the presence of glucose.

4. Isotope Experiments.—At 0 time, a known concentration of cells was mixed with a tagged K^+–Na^+ Ringer solution. Cell volume approximated 1 per cent of the total suspension volume after mixing. In some experiments, only the potassium was marked as K^{42}. In others, both potassium (K^{42}) and sodium (Na^{22}) were used simultaneously. Since only minute quantities were necessary for tracer studies and the Ringer's solutions were adequately buffered, no neutralization of the $K_2^{42}CO_3$ solution was carried out. Na^{22} was introduced as the chloride.

10 ml. samples were removed at time intervals into 15 ml. centrifuge tubes. 30 seconds to a minute's centrifugation at maximum speed in the International centrifuge (3000 R.P.M.) was sufficient to throw down the cells. The cells were washed once by resuspension in 5 per cent dextrose (or 10 per cent sucrose) and again recentrifuged for 30 seconds to a minute. The wash fluid was discarded and the cells brought to a final volume of 10 ml. with distilled water and then transferred to shell vials for counting. Residual contamination after washing was about 1 part in 5000 as determined by inulin dilution methods.

Counting was done in a well-type scintillation counter using a thorium-activated sodium iodide crystal. When K^{42} and Na^{22} were counted simultaneously, samples were counted immediately following the experiment and again a week later, when only Na^{22} remained. K^{42} counts were obtained by difference and corrected for decay.

For determination of cellular K^+ and Na^+, the counted samples were centrifuged to remove cell debris and the supernatant analyzed in the flame photometer. Results were expressed either on the basis of cell number or dry weight (3.6 mg. dry weight = 10^7 cells).

Fluxes were calculated using the equations described by Sheppard and Martin (9) for steady state conditions and also for conditions under which cell K^+ and Na^+ were changing.

RESULTS

Intracellular-Extracellular Distribution of Electrolytes.—

The electrolyte pattern immediately after removal of the tumor cells from the animal is shown in Table I. The cells were thrown down by centrifugation at 50 *G* for 1 minute and the supernatant removed for analysis. These values are listed under the column labeled Extracellular. The cells were then rinsed quickly with either 5 per cent dextrose or 10 per cent sucrose and then packed at 3000 *G* for 20 minutes. Results of analyses made on the packed cell mass

were calculated in terms of cell water and are listed under the column labelled Intracellular.

The intracellular to extracellular ratio for K^+ is 33, Na^+, 0.39, and Cl^-, 0.55. However, it is not enough to have knowledge of concentration gradients; one needs to know the electrochemical gradients across the cell membrane in order to compute the driving forces for electrolytes.

For nerve fibers, intracellular recording has given reliable figures, but for single cells successful recordings have been lacking. We are required then to use indirect methods for making this estimate. For the human erythrocyte, Solomon (11) has used the chloride distribution as a means for doing this, assuming that Cl^- does not participate in a carrier system, but distributes itself passively. For the red cell, this is a reasonable assumption; the evidence is considerable that the Cl^- is diffusible across the membrane (12).

TABLE I

Electrolyte Distribution between the Ehrlich Ascites Tumor Cell and Its Environment

	Intracellular	Extracellular
K	134 m.eq./liter cell water	4 m.eq./liter
Na	50 " " " "	128 " "
Cl*	64 " " " "	116‡ " "

* From Christensen *et al.* (7).

‡ From Albritton (10).

Conclusive evidence is not available for other mammalian cells. However, Wilson and Manery (13) have demonstrated a linear relation between cell chloride concentration and external chloride concentration in the polymorphonuclear leukocyte of the rabbit. Using their data, the author has calculated a potential difference of about 11 mv. cell interior negative to environment.

This type of study has not been reported for the ascites tumor cell. There is, however, an indirect piece of evidence for the hypothesis of passive movements of Cl^-; this is the demonstration of an exchangeability of Cl^- with another anion along diffusion gradients. The method for this demonstration measures osmotic phenomena and has been used by Parpart (14) to describe Cl^-–$SO_4^=$ exchanges in the erythrocyte.

When tumor cells were placed in a K^+–Na^+ Ringer solution in which the NaCl was replaced by an isosmotically equivalent amount of Na_2SO_4 (K^+–Na^+–$SO_4^=$ Ringer), the cells decreased in volume (Fig. 1). On the other hand, cells previously equilibrated in a K^+–Na^+–$SO_4^=$ Ringer solution, when returned to a K^+–Na^+ Ringer solution, were seen to swell.

The shrinkage of the cell in this type of experiment has been explained by the loss of a net amount of osmotically active material, specifically Cl^-, when

univalent Cl^- exchanges for divalent $SO_4^=$. The ideal exchange would be two Cl^- for one $SO_4^=$, but because of the differences in the basicity of the two ions, the exchange is less than two, but greater than one. That the cells equilibrated with $SO_4^=$ swell when placed in a Cl^--rich, $SO_4^=$free medium (K^+–Na^+ Ringer)

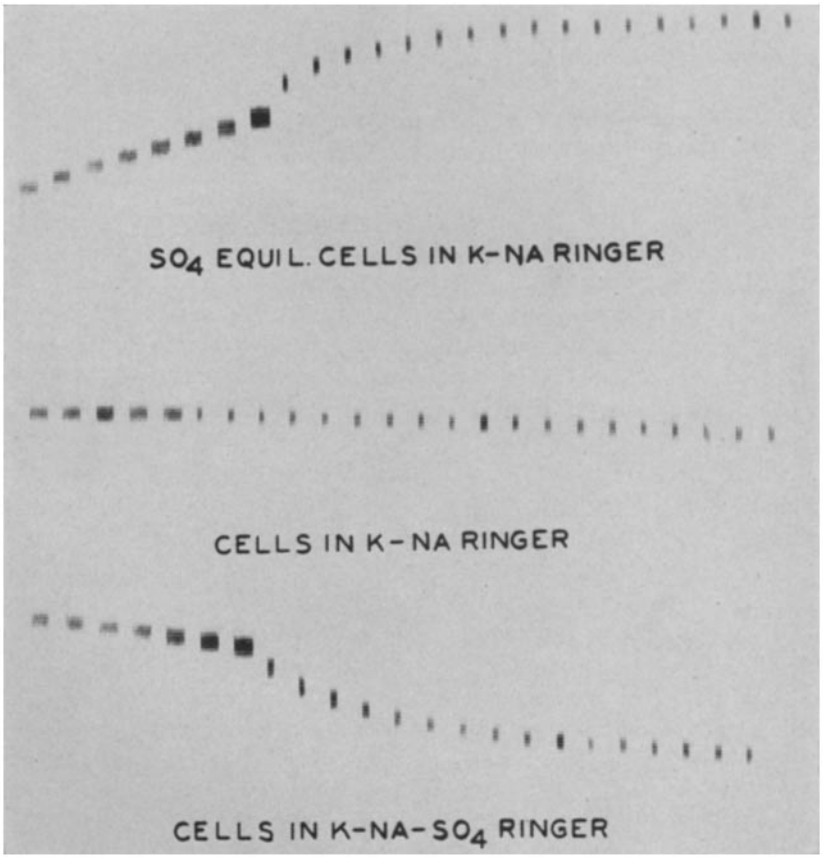

FIG. 1. Densimeter recordings of volume changes in the Ehrlich mouse ascites tumor cell during Cl^-–$SO_4^=$ exchanges. Upward deflections, increase in cell volume; downward deflection, decrease in cell volume. Wide bars indicate 15 second intervals; narrow bars indicate 1 minute intervals.

is evidence for the reversibility of the phenomenon. The rate at which the shrinking or swelling occurs provides an index of the rate at which the ion exchange is occurring.

Accepting then the existence of freely diffusible Cl^- across the tumor cell membrane, we can calculate the potential difference across the membrane for

subsequent use in describing electrochemical gradients. This value comes out to about 15 mv., cell interior negative to environment.

The Effect of Temperature.—

One of the first factors which was considered was the effect of temperature on the Na^+ and K^+ concentrations in these cells. Other cells, such as the human erythrocyte and the rabbit leukocyte have shown depletion of cell K^+ and replacement by external Na^+ when the temperature has been dropped. The rate of loss has varied with the different cell types. For the red cell, Ponder (15) finds 68 per cent of the K^+ has been lost from the cells after cold storage for 72 hours. The author (8) observed that the K^+ concentration in rabbit polymorphonuclear leukocytes dropped to half the original value in about 8 hours, at temperatures of 2°C., indicative of a marked sensitivity to low temperatures. A reciprocal increase in Na^+ concentration was also noted.

In these experiments, the tumor cells were washed several times with cold K^+–Na^+ Ringer's with glucose at 100 mg. per cent and then kept subsequently at temperatures of 2–5°C. in the same environment. Samples were taken off periodically and analyzed for Na^+ and K^+ and determinations made for per cent water.

The data have been handled with the following hypothesis in mind. It is conceived that low temperatures establish a system in which active processes are reduced. Net fluxes will be down electrochemical gradients. If one treats these net fluxes by first order kinetics, it is possible to obtain quantitative descriptions for the behavior of the membrane toward the two ions. These values will be minimum values since the existence of an active transport of Na^+ and K^+ in the opposite direction cannot be completely ruled out. This is especially so in these experiments in which, in the presence of glucose, some glycolysis and respiration are going on in spite of the reduced temperatures. Thus pH has been observed to drop from 7.42 to 7.21 during 20 hours at 2–5°C. Nevertheless, since both ions are being examined simultaneously, this error will not interfere with a comparison of the resistance which the membrane offers to the net movements of the two ions. By making such a comparison, one may estimate to what extent the two ions are linked in their movements.

Consider then the total exchangeable concentration of cation as $| B_0^+ - B_\infty^+ |$, in which B_0^+ is the cation concentration (milliequivalents/kilo cell water) at 0 time and B_∞^+ the cation concentration at exchange equilibrium. Similarly, $| B_t^+ - B_\infty^+ |$ is the exchangeable concentration in the cell at time, t. Then:

$$\frac{d|B_t^+ - B_\infty^+|}{dt} = P\frac{A}{V}|B_t^+ - B_\infty^+|$$

in which P = a permeability coefficient with the dimensions of centimeters/hour
A = surface area
V = volume of cell water

which gives on integration

$$\log \frac{|B_t^+ - B_\infty^+|}{|B_0^+ - B_\infty^+|} = \frac{PA}{2.3V} t$$

PA/V, with the dimensions of hr.$^{-1}$, may be designated as k, and is a measure of that fraction of exchangeable ion which is lost or gained per hour.

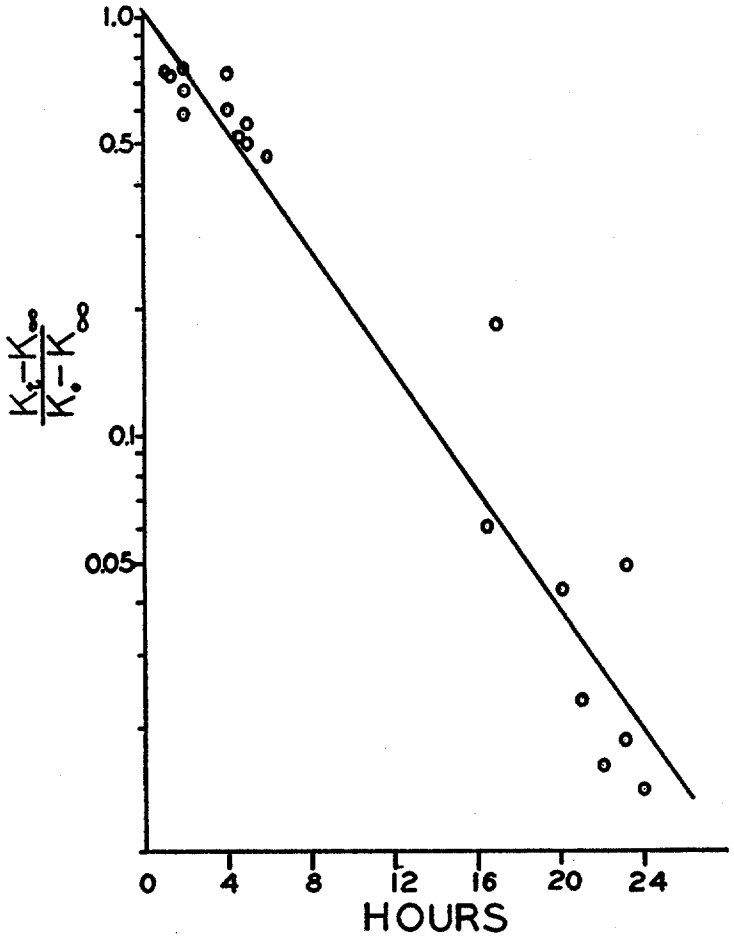

FIG. 2. Potassium loss from the Ehrlich mouse ascites tumor cell maintained at 2°–5°C. in the presence of glucose.

Since the concentration of cell K^+ tended toward the concentration of the external environment as an asymptote, it was decided to set $B_\infty^+ = B_e^+$ in which B_e^+ is the concentration of ion in the external environment. This remained

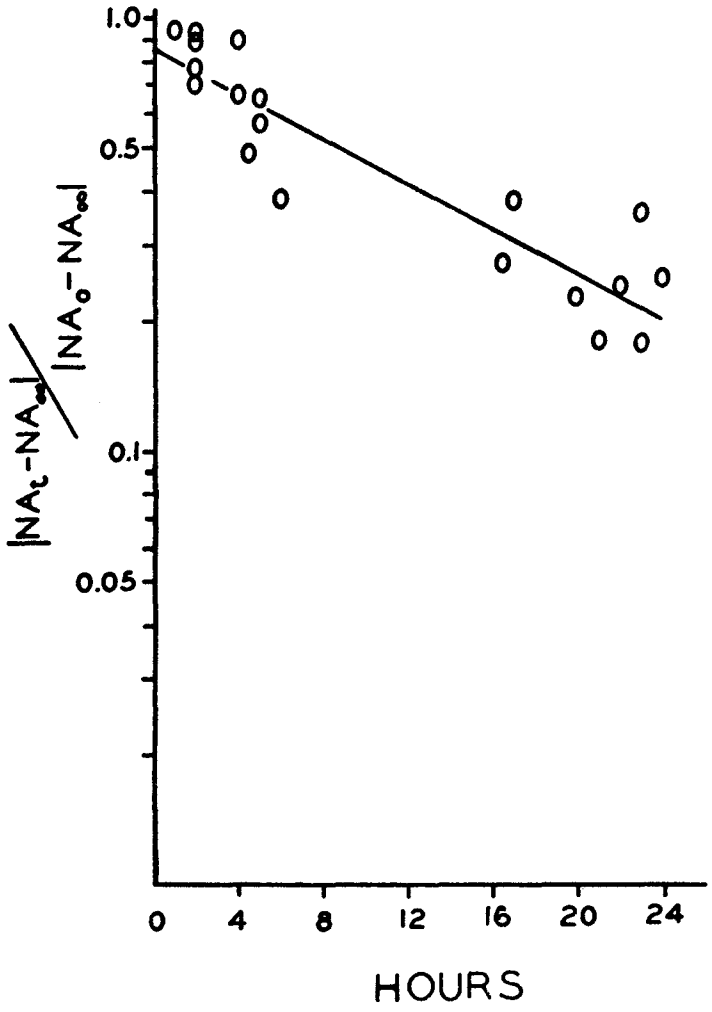

FIG. 3. Sodium gain in the Ehrlich mouse ascites tumor cell maintained at 2° to 5°C. in the presence of glucose.

constant since the cell volume approximated only 1 per cent of the total volume of the system.

Figs. 2 and 3 show plots of the log $\frac{|B_t^+ - B_e^+|}{|B_0^+ - B_e^+|}$ *vs.* t for the situations when

K^+ leaves the cell and when Na^+ enters. The use of absolute terms permits the plot of both ions in the same direction, although it must be remembered that K^+ is moving out while Na^+ is moving in.

Linear regression lines have been drawn for the two curves. $k = 0.157$ hr.$^{-1}$ and $P = 0.42 \times 10^{-4}$ cm./hr. for potassium loss while Na enters the cell with a $k = 0.058$ hr.$^{-1}$ and $P = 0.16 \times 10^{-4}$ cm./hr. Values for k between Na^+ and K^+, as well as values for P, differ significantly with a probability less than 0.01. At 0 time, values for $\log \frac{|B_t^+ - B_e^+|}{|B_0^+ - B_e^+|}$ do not differ significantly from 1.0 for either curve.

The data would serve to indicate that Na^+ and K^+ differ with respect to the resistance which the membrane offers to their net movements, and that their net movements under these conditions are not linked in any simple 1 to 1 relationship.[2]

Potassium Accumulation and Sodium Extrusion in the Presence of Glucose.—

Experiments were designed to assess the ability of the ascites tumor cell to move ions against electrochemical gradients. The approach was similar to that described by Harris (16) for the human erythrocyte.

Washed tumor cells were permitted to lose potassium and gain sodium at refrigerator temperatures in the presence of glucose, usually for an overnight period. At the start of the experiment, aliquots were resuspended in fresh K^+–Na^+ Ringer without glucose or with glucose, and placed either at 37°C., or kept at refrigerator temperatures (2–5°).

Table II summarizes the results from this type of experiment. The data have been expressed in terms of electrolyte amounts in milliequivalents/kilo dry weight. Since the water content of the cell increases as much as 79 per cent during the stay at low temperatures and then returns to normal when the temperature is 37°C., it was more advisable to use the dry weight as a common denominator for calculating the amounts of electrolyte which were gained or lost. To estimate electrochemical gradients, concentrations of electrolyte in intracellular water were used. The values shown for electrochemical gradients in Table II are from an experiment chosen at random.

Two conclusions may be drawn from these results. First, it is evident that K^+ is accumulated into the cell while sodium is extruded. However, and this is perhaps more useful information, the presence of glucose produces a seven-fold increase in the rate of K^+ accumulation without influencing the rate of extrusion of Na^+. Here again, is evidence for the divorcement of the two ion movements.

[2] Since the driving force for the loss of potassium and/or the gain of sodium is taken as the *exchangeable cell concentration* of the electrolyte, the electrical gradient across the membrane is not considered, and in this respect, the permeability coefficient, P, is limited in its applicability.

This conclusion is further substantiated by an experiment in which the temperatures to which the cells were returned were different. After remaining overnight at low temperatures, cells were returned to environments of 22° and 37°C. respectively and given glucose at 100 mg. per cent. The Q_{10} for K^+ accumulation was 2.2; the Q_{10} for Na^+ was 1.1.

During the accumulation-extrusion processes, H^+ is being produced in considerable amounts as glycolysis proceeds. Rates of 5.0 $\mu M/10^7$ cells/hr. have been observed, based upon changes in total titratable H^+. These values

TABLE II

Accumulation of Potassium and Extrusion of Sodium at 37°C. in the Presence and Absence of Glucose

With glucose		Without glucose	
K^+/kg. dw/hr.	Na^+/kg dw/hr.	K^+/kg. dw/hr.	Na^+/kg. dry wt./hr.
140 ± 25 (6 experiments)	460 (5 experiments)	22 ± 7 (3 experiments)	460 (3 experiments)

Electrochemical gradients	
For potassium accumulation	For sodium extrusion
Initial intracellular, 7.8 m.eq./kg. cell water	143 m.eq./kg. cell water
" extracellular, 6 m.eq./liter	173 m.eq./liter
Gradient, −140 cal./mol	+400 cal./mol
After 1 hr. and 40 min. at 37°C.	
Intracellular, 62 m.eq./kg. cell water	93 m.eq./kg. cell water
Extracellular, 5.8 m.eq./liter	175 m.eq./liter
Gradient, +1000 cal./mol	+700 cal./mol

agree well with data of Kun et al. (5) who reported 5.7 $\mu M/10^7$ cells/hr. of lactic acid being produced by cells undergoing aerobic glycolysis. It would appear that most of the H^+ being produced leaves the cell with lactate anion, so that a H^+ for K^+ exchange from glycolysis is not a basic mechanism. On these grounds alone, this conclusion is not unequivocal because of the small K^+ accumulation (0.50 $\mu M/10^7$ cells/hr.) relative to the lactic acid production (5.7 $\mu M/10^7$ cells/hr.). An amount of H^+ exchanging for K^+ may not be detected as such in chemical determinations of lactate ion. Subsequent evidence, however, from net fluxes during isotope experiments carried out in the absence of glucose and glycolysis, will support the hypothesis that the K^+–H^+ exchange so characteristic of yeast cells (17, 18) is not operative in the ascites tumor cell.

Measurements of Potassium and Sodium Fluxes.—

At this stage of the investigation, because of the glucose effect it was believed that K^+ accumulation was probably associated with glycolysis although not through a H^+ for K^+ exchange. This view was changed radically when studies were conducted on environments free of an exogenous source of glucose.

Isotope studies were undertaken to determine exchange rates in the steady state. Room temperatures were chosen to start off the investigation because rapid exchanges were anticipated from the net flux studies reported above. It was decided also to avoid the complications of H^+ production and pH changes by leaving out the glucose. But from some inexplicable belief that everything stays better at low temperatures, the cells were washed at low temperatures, (7–10°C.), and kept at 2–5°C. until ready for the isotope experiment at 22°C. The following flow sheet describes the experimental setup.

Aspirated cell suspension
↓
Washed three times with K^+–Na^+ Ringer without glucose
↓
Resuspend in K^+–Na^+ Ringer without glucose and
↓
determine cell count of the suspension.
↓
Keep in refrigerator until mixing time (2–5°C.)

} 7–10°C.

At 0 time:

Cell suspension + K^{42}–Na^{22} Ringer without glucose
Mix at 22°C.
↓
Sample at various intervals

The first striking observation was the level of potassium and sodium in the cells after these relatively brief periods of exposure to low temperatures in the absence of glucose. Under these conditions electrolyte changes occur very rapidly. For example, after 120 minutes (Table III), there is only 27 per cent of the exchangeable potassium remaining in the cell while 64 per cent of the exchangeable sodium has entered the cell. Reference to Figs. 2 and 3 indicates that when glucose is present, a comparable potassium loss would require 8 hours and a comparable sodium gain would require *ca.* 14 hours.

The second observation was that when the cell suspensions were raised to 22°C. for measurements of their exchange rates, net accumulation of K^+ and net extrusion of Na^+ occurred even though there was no glucose available exogenously. This observation would rule out the necessity for glycolysis as a source for the metabolic energy of the ion transfers, since Kun *et al.* (5), as well as McKee *et al.* (4), have shown that glycolysis is measurable only in the presence of exogenous glucose. It is true that glucose was necessary for the

accumulation of K^+ after a prolonged period at low temperatures, while Na^+ could be extruded in the absence of glucose (*cf.* Table II). It could be argued, however, that over a prolonged period the supply of metabolite necessary for K^+ accumulation has been used up and that glucose is required in order to replenish the supply, while Na^+ can be extruded with what is available. Additional evidence for the dependence of the potassium accumulation on the internal metabolite level comes from observations on the influx of radioactive-labelled potassium into cells which had been at 22°C. from the time of removal of the cells from the animal and had not been given a source of exogenous

TABLE III

Exchange Rates for the Ehrlich Ascites Tumor Cell, Using K^{42} and Na^{22}

Experiment	Time at 10°C. or less	Electrolyte level at time of mixing*		Fluxes measured at 22°C.‡		
	min.	K^+	Na^+	*Influx*	*Net*	*Efflux*
Aug. 15, 1956	0	95	35	0.82	0	0.82 K
				—	—	— Na
Feb. 6, 1957	0	124	27	0.82	0	0.82 K
				0.77	0	0.77 Na
July 3, 1956	38	79	60	1.4	0.08	1.4 K
				—	−0.16	— Na
July 2, 1956	90	75	80	1.1	0.14	0.98 K
				—	—	— Na
Aug. 7, 1956	103	53	90	3.2	0.46	2.5 K
				1.0	−0.24	1.2 Na
July 30, 1956	120	41	128	1.7	1.3	0.42 K
				—	−1.4	—

* Milliequivalents/kilo cell water.

‡ Millimols/10^7 cells/hour. To convert to milliequivalents/kilo dry weight/hour multiply by $10^3/3.6$.

metabolite (Fig. 4). During the course of these experiments, the net flux was 0. However, it has been observed that the rate constants for influx assume different and lower values and that this change in rate becomes apparent at about 150 minutes from the time of removal of the cells from the animal. Either one is seeing the appearance of a new compartment in these starved cells or else the transport mechanism is shifting to a new, lower level.

Both the net accumulation rate of potassium and the net extrusion rate of sodium which occurred when the cells were shifted from 2° to 22°C. increased with increased time that the cells were at the low temperatures prior to the shift (Table III). This correlates with the levels of K and Na intracellularly; when the initial electrochemical gradient for the ion was low, the net accumulation rate for the ion was high and *vice versa*.

The net fluxes observed for the ions in most of the experiments were usually

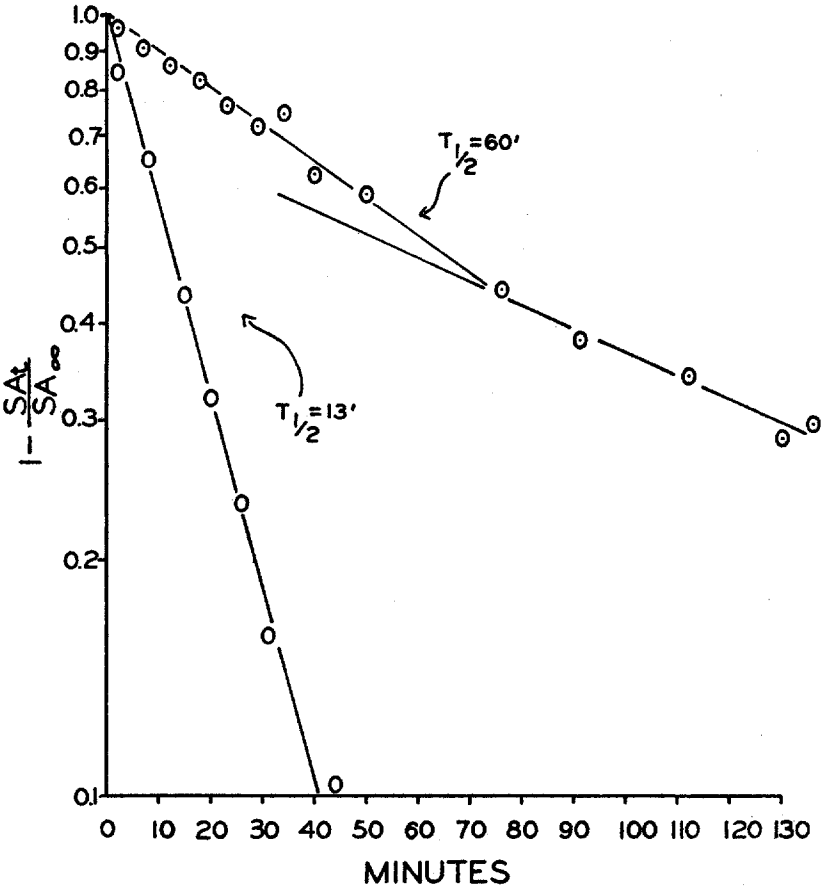

FIG. 4. Influx of potassium ion at 22°C. into washed Ehrlich mouse ascites tumor cells against an electrochemical gradient (μM/10^7 cells/hour).

⊙, at 22°C. throughout washing and experimental procedures.

Influx = 0.82
Net flux = 0
Efflux = 0.82
Gradient = $+1.2 \times 10^3$ cal./mol.

○, preliminary exposure to low temperatures for 103 minutes.

Influx = 3.2
Net flux = 0.46
Efflux = 2.7
Gradient = $+0.9 \times 10^3$ cal./mol.

not more than 15 per cent of the exchange rate. Thus one could get good agreement between influxes calculated by Sheppard's steady state equation and by his more general equation for systems in which there was a net flux (9). The values given in the table are calculated from the more general equation, and made at the point of maximum net flux change. However, as seen from Fig. 4 for example, one could utilize the steady state equation equally well.

Fig. 4 serves to illustrate another general observation for these cells. The exchange rates for K^+ are considerably greater when the cell is shifted from 2° to 22°C. than when the cell is maintained at 22°C. throughout the preparatory stages prior to measurement.

TABLE IV

Response of Exchange Rates to a Shift in Temperature in the Absence of Exogenous Glucose

	Influx	5°C*	20°C.‡	Q_{10}
				μM/10⁷ cells/hr.
K^+		0.22	2.6	5.6
Na^+		3.7	5.0	1.2
	Efflux			
K^+		0.22	1.8	4.1
Na^+		3.0	5.7	1.2
	Net Flux			
K^+		0	+0.8	—
Na^+		+0.72	−0.71	1.6

* Measured at 20 minute point, where net rate inward for sodium was maximum.

‡ Measured at 8 minute point following shift to new temperature.

To analyze this observation in greater detail, an experiment was designed to measure the exchange rates for the two ions at the low temperatures and then to shift to the higher temperature and measure the fluxes at that temperature.

Two aliquots of a known concentration of washed cells were prepared at 7–10°C. One aliquot was mixed with a tagged solution of K^+–Na^+ Ringer's without glucose and periodic sampling carried on at temperatures of 2.5–4.5°C. After 47 minutes, the second aliquot which had been at these same low temperatures was mixed with a tagged K^+–Na^+ Ringer's solution at 20°C. and brought to this temperature in less than a minute. Periodic sampling was then carried out at this temperature.

The results are summarized in Table IV and Fig. 5. To convert from milliequivalents/kilo dry weight to $\mu M/10^7$ cells/hr. multiply by $10^3/3.60$. From Fig. 5, it is evident that the period of washing at low temperatures (36 minutes prior to the first measurement) in a K^+–Na^+ Ringer solution without glucose has drastically reduced the K^+ level in the cells. On a cell water basis, this

new K^+ level is about 34 m.eq./kilo cell water. The Na^+ level, however, changes more gradually during the stay at low temperatures. Transfer to 20°C. as indicated, reverses the net fluxes and K^+ is accumulated as Na^+ is extruded. It would appear from the rates of accumulation and extrusion that the two systems are linked. If they are, it is only through their net fluxes, for the

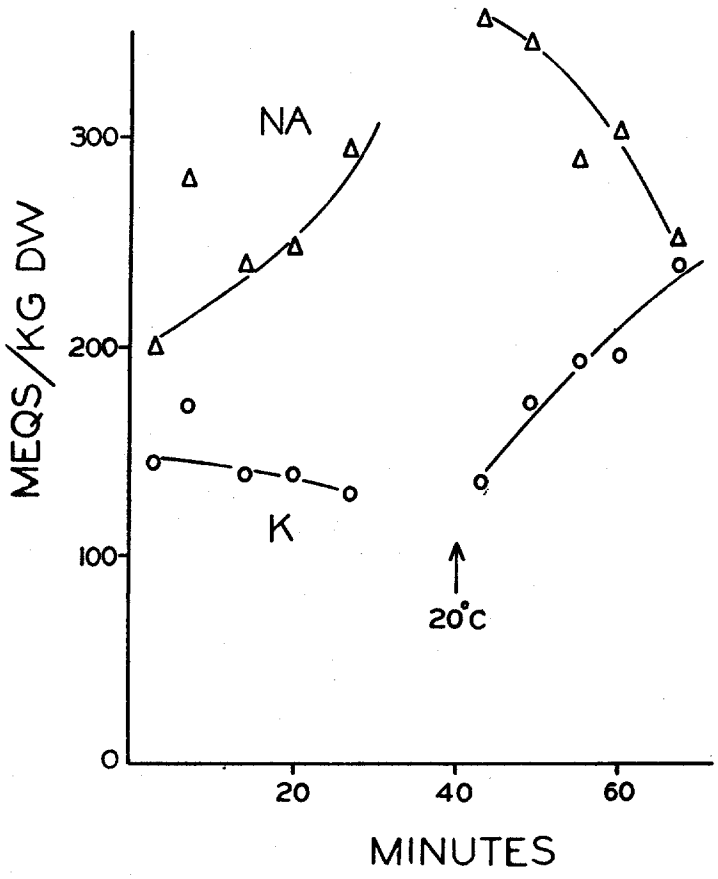

FIG. 5. Changes in the net fluxes of potassium (○) and sodium (△) in the Ehrlich mouse ascites tumor cell in response to a shift in temperature from 2° to 22°C.

response of the exchange rates for the two ions differs markedly. From Table IV, it is seen that a shift in temperature produces an extremely large increase in the exchange rate for potassium, with a Q_{10} of more than 4; sodium ion is increased only slightly relative to the effect on potassium. It is important to realize, also, that the net fluxes which one observes with the change in temperature are not attributable to the effect on only one of the fluxes, but that both influx and efflux for each of the ions are increased.

DISCUSSION

The observations which have been reported indicate that there are separate transport mechanisms for sodium and potassium in the Ehrlich ascites tumor cell. Thus it has been shown that at low temperatures with a diminished metabolism, net fluxes for the two ions are different. We have seen also, that the cell can accumulate potassium and extrude sodium against electrochemical gradients for each ion. Moreover, it has been possible again to separate the two ions by several additional criteria, such as the influence of glucose on the accumulation of potassium following prolonged refrigeration and the demonstration that potassium exchange rates are temperature-dependent, while exchange rates for sodium are temperature-insensitive.

Some estimate can be made as to the nature of the metabolism which is associated with these ion transfers. That one gets an accumulation of potassium and an extrusion of sodium in the absence of an exogenous source of glucose is evidence for dismissing glycolysis as a primary metabolic mechanism, for Kun *et al.* (5) as well as McKee and his associates (4) have shown that glycolysis occurs only in the presence of an exogenous source of glucose.

It may be equally constructive to turn attention to changes which occur not in the net fluxes but in the exchange rates; *i.e.*, the influx-efflux for each ion. The net fluxes which occur when the cells are shifted from temperatures of 2–5°C. to a temperature of 22°C. are associated with an increase in the over-all exchange rate with the influx for potassium, for example, increasing more than the efflux for the same ion, resulting in a net flux inward. For the potassium ion, in relative terms, this increase in exchange rate is quite large; for sodium ion, the increase is minimal.

Using the approach suggested by Davies (19), we can express the difference in influx at 5°C. and 20°C. in microliters/milligram dry weight/hour for comparison with oxygen consumption figures. Using the figures from Table IV, for potassium this value is 15 μl./mg. dry weight/hr.

Since oxygen consumption studies were not carried out in this set of experiments, we must rely upon the published results under as comparable a set of experimental conditions as are available in the literature. Chance and Castor (20) have reported Q_{O_2} values of 3 μl./mg. dry weight/hr. for cells which were prepared in the cold and measured subsequently at 25°C. Using this data, the ratio of ion transport to oxygen consumption is 5. If we choose the higher value for oxygen consumption of 8 μl./mg. dry weight/hr. obtained at 37°C. (4, 5), the ratio is still greater than 1. Thus for potassium influx, estimates of this sort permit one to eliminate at least two metabolic mechanisms, namely, those which would require the production of organic acids such as lactic or pyruvic acid in which the ratio could not be greater than 0.33 and those dependent upon the production of carbon dioxide or carbonic acid in which the

ratio could not exceed 1 (19). A similar case can be established for sodium efflux (Table IV) in which ratios greater than 1 are also obtained.

There are two biochemical systems which do predict ratios of ion transport to oxygen consumption greater than 1. One involves the passage of electrons along the cytrochrome system. In this system the ratio cannot exceed 4. The other system involves the production of high energy phosphate bonds, such that with P/O ratios of 3 to 1, the ratio of ions moved to oxygen consumed cannot exceed 6, if one ion is moved for every high energy P formed (19).

Both these systems are available in the Ehrlich ascites tumor cell. Chance and Castor (20) have shown the existence of cytochromes *a*,*c*, and a_3 in amounts comparable to amounts found in systems like yeast cells and heart muscle preparations. They note, however, that cytochrome *b* is conspicuous by its very low concentrations while cytochrome *c* is present in unusually large amounts relative to other cell and homogenate systems. And yet in their paper in 1956, Chance and Hess (2) use cytochrome *b* to elucidate changes in the ADP level in these cells. From a functional point of view, it would appear that the cytochrome system is intact in these cells.

With regard to the existence of oxidative phosphorylation, Lindberg, Ljunggren, Ernster, and Revesz (21) have measured P/O ratios for the mitochondria of the Ehrlich ascites tumor cell. They report values which are somewhat lower than those seen in other cell systems.

Energetics of Potassium and Sodium Transport.—

The energy expenditures supporting the rapid fluxes observed merit some attention. Even though the cell system may not have gained or lost free energy, on the basis of reversible thermodynamics, in exchanging one ion for itself, as, for example K^+ for K^+, or Na^+ for Na^+, when the net flux is 0, nevertheless, the ability of the exchange so described to continue at a state above the equilibrium requires the expenditure of energy and at a rate compatible with the exchange rate observed.

For the steady state situation, at 22°C. (Fig. 4 and Table III), the exchange rates for both Na^+ and K^+ are approximately 0.8 μM/10^7 cells/hr. From a ratio of $K_i/K_e = 20.3$ and an electrical gradient of 15 mv. negative inside to outside, the electrochemical gradient for potassium calculates out as 1400 cal./mol, so that the required rate of energy expenditure was 1120×10^{-6} cal./10^7 cells/hr. If we use the Q_{O_2} values quoted by Chance and Castor (20), which are probably minimal for this temperature, the energy equivalent for complete oxidation to CO_2 and water is 324×10^{-3} cal./10^7 cells/hr. This would mean that only 0.3 per cent of the available energy from oxidative metabolism would be used for an irreversible transport of potassium. Since the influx and efflux for this ion may be coupled in an exchange diffusion type of system, this value is a maximum.

For sodium exchange, in which the ratio $Na_i/Na_e = 0.162$ and the electrical gradient is 15 mv. negative inside to outside, the electrochemical gradient is also 1400 cal./mol so that for sodium transport the same percentage of the available energy from oxidative metabolism would be used. If the two transport systems are unlinked, the total expenditure of energy would still not require more than approximately 0.6 per cent of the energy available to the cell.

It must be noted, however, that the possibility exists that in the steady state at 22°C. the two transport systems may be linked so that the energy expenditure under these conditions may be less. The fact that the electrochemical gradients for the two ions are identical and that their exchange rates are identical gives some support to this possibility. However, this link is not obligatory for, as we have demonstrated above, one can devise experimental situations which will separate the two transport systems.

TABLE V

Conversion Table

From	To	Multiply by
1. $\mu M/10^7$ cells/hr.	μliters/mg. dry weight/hr.	6.2
2. $\mu M/10^7$ cells/hr.	mM/kg. dry weight/hr.	$10^3/3.6$
3. μliters/mg. dry weight/hr.	M.eq./kg. dry weight/hr.	$10^3/22.4$
4. $\mu M/10^7$ cells/hr.	$pM/cm.^2/sec.$	$10^3/270$
5. Millivolts of electrochemical gradient	Calories/mole	23

The spherical shape of the ascites tumor cell permits an easy calculation of its surface area and from this and flux data, the flux may be expressed in dimensions of $pM/cm.^2/sec$. The mean surface area for this cell is 750 μ^2 and for a steady state flux of 0.82 $\mu M/10^7$ cells/hr., the flux is calculated to be 3.0 $pM/cm.^2/sec$.

To allow the reader to move conveniently from one set of dimensions to another, Table V has been included. Conversion factors have been listed which are applicable to the Ehrlich ascites tumor cell in order to permit ready comparison with other cells.

BIBLIOGRAPHY

1. Christensen, H. N., and Riggs, T. R., *J. Biol. Chem.*, 1952, **194,** 57.
2. Chance, B., and Hess, B., *Ann. New York Acad. Sc.*, 1956, **63,** 1008.
3. Racker, E., *Ann. New York Acad. Sc.*, 1956, **63,** 1017.
4. McKee, R. W., Lonberg-Holm, K., and Jehl, J., *Cancer Research*, 1953, **13,** 537.
5. Kun, E., Talalay, P., and Williams-Ashman, H. G., *Cancer Research*, 1951, **11,** 855.
6. Christensen, H. N., Riggs, T. R., Aspen, A. J., and Morton, S., *Ann. New York Acad. Sc.*, 1956, **63,** 983.

7. Christensen, H. N., Riggs, T. R., Fischer, H., and Palatine, I. M., *J. Biol. Chem.*, 1952, **198,** 17.
8. Hempling, H. G., *J. Cell. and Comp. Physiol.*, 1954, **44,** 87.
9. Sheppard, C. W., and Martin, W. R., *J. Gen. Physiol.*, 1950, **33,** 703.
10. Albritton, E. C., Standard Values of Blood, Philadelphia, W. B. Saunders Co., 1952.
11. Solomon, A. K., *J. Gen. Physiol.*, 1952, **36,** 57.
12. Henderson, L. J., Blood: A Study in General Physiology, New Haven, Yale University Press, 1928.
13. Wilson, D. L., and Manery, J. F., *J. Cell. and Comp. Physiol.*, 1949, **34,** 493.
14. Parpart, A. K., *Cold Spring Harbor Symp. Quant. Biol.*, 1940, **8,** 25.
15. Ponder, E., *J. Gen. Physiol.*, 1950, **33,** 745.
16. Harris, J. E., *J. Biol. Chem.*, 1941, **141,** 579.
17. Rothstein, A., and Enns, L. H., *J. Cell. and Comp. Physiol.*, 1946, **28,** 231.
18. Conway, E. J., and O'Malley, E., *Biochem. J.*, 1946, **40,** 59.
19. Davies, R. E., *Active Transport, Symp. Soc. Exp. Biol. (Great Britain)*, 1954, **8.**
20. Chance, B., and Castor, L. W., *Science*, 1952, **116,** 200.
21. Lindberg, O., Ljunggren, M., Ernster, L., and Revesz, L., *Exp. Cell Research*, 1953, **4,** 243.