SODIUM AND POTASSIUM FLUX RATES IN NORMAL HUMAN LEUCOCYTES IN AN ARTIFICIAL EXTRACELLULAR FLUID

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SUMMARY

1. Sodium and potassium efflux and influx rates were studied in normal human leucocytes in an artificial extracellular fluid using radioactive isotopes.
2. The rate constant for sodium efflux was $4.2 \text{ h}^{-1}$ corresponding to a sodium efflux rate of $487 \text{ mmol kg cell dry weight}^{-1} \text{ h}^{-1}$. Approximately three-quarters of this flux was ouabain sensitive.
3. Potassium influx was $346 \text{ mmol kg cell dry weight}^{-1} \text{ h}^{-1}$. Approximately two-thirds of the potassium influx was ouabain insensitive.

Key words: sodium, potassium, flux, leucocytes.

It is generally accepted that the high concentration of potassium and the low concentration of sodium found in most living cells is maintained by an ATP-dependent transport system located in the cell membrane. The intracellular concentrations of these ions are determined by the activity of the transport system, the sodium pump and passive movement of the ions down electrochemical gradients. Much of our understanding of the processes involved has come from investigation of the erythrocyte. However, the erythrocyte is unrepresentative of mammalian cells as a whole because of its low rates of metabolism and ionic flux (Table 1). In these respects the leucocyte may be a more satisfactory model since it is nucleated and appears to have a less specialized metabolism than the erythrocyte.

Recently the leucocyte has been used as a method of investigating intracellular electrolyte and water composition under experimental conditions (Patrick & Bradford, 1972) and in disease states (Patrick, Jones, Bradford & Gaunt, 1972). In these studies, important differences between leucocyte and erythrocyte electrolyte composition were demonstrated. Further elucidation of the problems in this area appeared to require a knowledge of sodium and potassium transport rates in the leucocyte and the present paper is a report of experiments on human leucocytes in vitro in which flux rates of sodium and potassium were determined using radioactive isotopes.

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MATERIALS AND METHODS

Each of the studies to be described was performed on leucocytes derived from 50 ml of heparinized venous blood taken from healthy members of the hospital and laboratory staff. Leucocytes were prepared from the blood by the method of Baron & Ahmed (1969). Heparinized blood (15 ml) was mixed with 4 ml of tissue culture fluid (T.C. 199, Burroughs Wellcome) containing 6% Dextran T 250 (Pharmacia Ltd). T.C. 199 is a complex solution containing inorganic salts, amino acids, lipids, glucose, vitamins and other substances with an osmolality of 290 mosmol kg⁻¹. The final sodium concentration was 135–137 mmol l⁻¹. The mixture was allowed to stand until most of the erythrocytes had settled. The leucocyte-rich supernatant was then separated and spun at 160 g for 5 min. Residual erythrocytes were lysed by suspending the leucocyte plug in a hypo-osmolar solution for 13 s. The viability of the cell preparation was >95% as estimated by Trypan Blue exclusion. The final cell preparation was suspended in T.C. 199 at a pH between 7-35 and 7-45 and an osmolality between 280 and 290 mosmol kg⁻¹.

**Table 1. Estimates of sodium transport rates in various tissues. The value for the leucocyte is calculated on the assumption that the leucocyte is a sphere of radius 5 µm**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>10⁻¹²× Na efflux</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog muscle</td>
<td>14</td>
<td>Levi &amp; Ussing (1948)</td>
</tr>
<tr>
<td>Nerve fibre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>31–39</td>
<td>Hodgkin &amp; Keynes (1955)</td>
</tr>
<tr>
<td>Stimulated at 100 impulses/s</td>
<td>700</td>
<td>Keynes (1951)</td>
</tr>
<tr>
<td><em>Necturus</em> renal tubules</td>
<td>62</td>
<td>Oken, Whittenberg, Windhager &amp; Solomon (1963)</td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td>0·02–0·04</td>
<td>Calculated from Glynn (1956)</td>
</tr>
<tr>
<td>Human leucocytes</td>
<td>6</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Sodium efflux

The leucocyte suspension was incubated at 37°C with 5 µCi of ²²NaCl for 20 min. The cells were then separated by centrifugation at 160 g for 3 min and the supernatant was removed from the cell plug as completely as possible. The cells were washed once with 10 ml of T.C. 199 then resuspended in a further 10 ml of tissue culture fluid and returned to the water bath at 37°C. At various times up to 20 min, aliquots of the cell suspension were transferred to specially formed polythene tubes (Baron & Ahmed, 1969), immersed in an ice bath and centrifuged immediately at 0°C for 2 min at 160 g. The supernatant was removed as completely as possible by pipetting and then drying the inside of the tube with tissue. The cell plugs and aliquots of the final supernatant samples were counted in a well-type scintillation counter and scaler (Tracerlab). Specimens were timed for a minimum of 10⁴ counts. Specimen dry weight, determined after heating the specimens to constant weight at 100°C, was in the range 1·8–4·7 mg.
For each subject, sodium efflux was also studied in the presence of ouabain at a concentration of 10 μg ml⁻¹.

**Sodium influx**

The leucocyte suspension in tissue culture fluid at 37°C was prepared as previously described. At zero time, 5 μCi of ²²NaCl was added to the suspension. After incubation for 7.5 min, the suspension was centrifuged at 0°C for 1 min at 160 g. The supernatant was removed as completely as possible and the cells washed in 10 ml of ice-cold tissue culture fluid. After centrifugation in the cold for a further minute, the cell plug was again prepared as free as possible of supernatant. The cell plug and aliquots of the two supernatant specimens were counted for radioactivity as described above.

In these experiments, radioactive sodium influx effectively ceased at the time during centrifugation at which the cooled cells came together as a plug in the polythene tube thereby producing a micro-environment separate from the main mass of the extracellular phase. The time at which radioactive sodium influx stopped cannot be known with complete certainty but was taken to be 8 min from the start, i.e. 30 s after the start of centrifugation.

**Potassium influx**

The cell suspension was prepared as described above and, at zero time, 10 ml of T.C. 199 containing 20 μCi of ⁴²KCl at 37°C was added. Incubation was continued at 37°C. Samples were removed at times up to 28 min and immediately cooled in an ice bath, at which point active potassium influx was assumed to have stopped. The specimens were immediately centrifuged at 0°C for 1 min and the supernatant removed as completely as possible. The cells were then washed once in 10 ml of ice-cold T.C. 199 and again rendered as free as possible of supernatant by careful drying with tissue. The cell specimen and aliquots of the two supernatant specimens were counted in a well-type scintillation counter and scaler. The extracellular potassium in these experiments was in the range 5.8-6.2 mmol ¹⁻¹. Experiments were also performed in which influx of ⁴²K was studied in the presence of ouabain at a concentration of 10 μg ml⁻¹.

**Potassium efflux**

The cells were loaded with ⁴²K by incubation at 37°C for 40 min with 20 μCi of ⁴²KCl. The specimen was centrifuged for 2 min at 160 g, the supernatant removed as completely as possible and the cells washed once with 10 ml of T.C. 199. After resuspension in a further 10 ml of tissue-culture fluid, they were returned to the water bath at 37°C. Samples were removed at various times up to 40 min, cooled in an ice bath, centrifuged at 0°C and separated from the supernatant as rapidly as possible.

²²NaCl was obtained from The Radiochemical Centre, Amersham, England. ⁴²K₂CO₃ was obtained from the Atomic Energy Research Establishment, Harwell, England, and was converted into KCl by the addition of an appropriate quantity of HCl.

Sodium and potassium in the extracellular phase were estimated by means of a flame photometer (Instrumentation Laboratories Inc.).

It will be noted that in these experiments only one wash was used to free the leucocyte of contaminating radioactive extracellular fluid. This contrasts with the common practice in erythrocyte flux experiments of washing the cell sample several times which is possible because
of the low transport rate of the erythrocyte. The volume of the extracellular phase in our studies was approximately 100–200 times that of the cells and, if we assume the high value of 50% for the contamination of the centrifuged cell specimen with extracellular phase, a single wash will remove all but an insignificant proportion of contaminating radioactivity. In every experiment an aliquot of the final supernatant was counted and, assuming a contamination of 50%, there would never have been an error from this source of as much as 1%.

RESULTS

**Sodium efflux**

The sodium efflux rate-constant for the leucocyte was calculated from the regression line of the natural logarithm of radioactivity unit dry weight$^{-1}$ on time, which appeared to be a linear function. Observations were made on twenty normal subjects. The value obtained for the efflux rate constant ($k$) was 4.2±0.3 h$^{-1}$. In the presence of ouabain (10 µg ml$^{-1}$) the rate constant for sodium efflux was 1.0±0.1 h$^{-1}$.

Intracellular sodium concentration was not measured in the specimens used for the flux experiments but, in our laboratory, the mean value for leucocyte intracellular sodium concentration in normal subjects at room temperature is 32 mmol kg cell wet weight$^{-1}$ or 116 mmol kg$^{-1}$ cell dry weight. If these results are combined with the value obtained for the efflux rate constant, the sodium efflux rate becomes 134 mmol kg cell wet weight$^{-1}$ h$^{-1}$ or 487 mmol kg cell dry weight$^{-1}$ h$^{-1}$.

**Sodium influx**

From a knowledge of the specific radioactivity of the sodium in the extracellular phase in these experiments, it is possible to calculate the true rate of sodium influx ($m_t$) by substitution in the following equation provided the rate constant for sodium efflux is known.

\[
m_t = \frac{kx}{1 - e^{-kt}}
\]

Where $x$ is the observed sodium uptake (mmol kg cell dry weight$^{-1}$) and $t$ is the time in hours (0.133 in the present experiments).

Experiments on leucocytes from twelve normal subjects gave a value for sodium influx of 359±37 (SEM) mmol kg cell dry weight$^{-1}$ h$^{-1}$.

**Potassium efflux**

Over the period of 40 min used in these experiments, potassium efflux appeared to follow first-order kinetics and the rate constant was calculated from the relationship between ln counts remaining in the cell specimen and time as described for sodium efflux. The mean value for the efflux rate constant from eighteen observations on leucocytes from six normal subjects was 0.85±0.09 h$^{-1}$. If this value for the rate constant is coupled with the normal value for leucocyte intracellular potassium obtained in our laboratory (102 mmol kg cell wet weight$^{-1}$ and 367 mmol kg cell dry weight$^{-1}$), the efflux rate for potassium in these experiments was 87 mmol kg cell wet weight$^{-1}$ h$^{-1}$ or 312 mmol kg cell dry weight$^{-1}$ h$^{-1}$.

**Potassium influx**

Potassium influx was calculated from the observed uptake of $^{42}$K in six normal subjects using the equation given in the section on sodium influx to correct for the loss of radioactivity.
from the cells during the experiment. The mean value for potassium influx was 346±21 (SEM) mmol kg cell dry weight$^{-1}$ h$^{-1}$.

In the experiments in which the uptake of radioactive potassium was studied in the presence of ouabain, the influx rate was 245±27 (SEM) mmol kg cell dry weight$^{-1}$ h$^{-1}$.

**DISCUSSION**

In recent years, the mechanisms responsible for maintaining the gradients of sodium and potassium between cells and their external environment have been intensively studied. The erythrocyte, because of its ready availability and robustness *in vitro*, has been a natural choice for workers in this field and continues to yield information both in physiological studies and in disease states. However, the erythrocyte is a highly specialized cell, devoid of a nucleus, containing a large quantity of a single protein, haemoglobin, and having a slow and almost entirely anaerobic metabolism. Ionic flux determinations on cells other than the erythrocyte have tended to confirm what a uniquely inactive cell the erythrocyte is in this respect (see Table 1).

The leucocyte shares with the erythrocyte easy availability and the fact that it exists naturally not as an organized tissue but as a cell suspension. The study of leucocyte water and electrolytes in uraemia and potassium depletion has yielded results which differ qualitatively or quantitatively from those obtained with the erythrocyte (Patrick & Bradford, 1972; Patrick *et al.*, 1972).

The implication from these experiments was that the leucocyte was in a state of ionic flux considerably more active than the erythrocyte, a suggestion which has been confirmed by the present experiments.

The rate constant for sodium efflux, 4.2, contrasts with the value obtained for the erythrocyte of approximately 0.3 (Glynn, 1956). The fact that the leucocyte has a higher intracellular sodium content than the erythrocyte makes the disparity between the efflux rates for sodium even more striking—134 mmol kg cell wet weight$^{-1}$ h$^{-1}$ for leucocytes against 3 mmol kg cell wet weight$^{-1}$ h$^{-1}$ for erythrocytes. It is evident that the sodium transport rate in the leucocyte is some forty to fifty times that of the erythrocyte. The ouabain insensitive portion of leucocyte sodium efflux at approximately 36 mmol kg cell wet weight$^{-1}$ h$^{-1}$ is again much greater than the corresponding value for the erythrocyte (1 mmol kg cell wet weight$^{-1}$ h$^{-1}$) but is a slightly smaller proportion of the total sodium efflux.

In an active system such as leucocytes, the determination of sodium influx presents problems since the time taken to perform the various manipulations involved in terminating the experiment is large compared with the length of the study itself. The time of 8 min was chosen for these experiments as a result of preliminary studies in which sodium influx was observed sequentially in the same cell suspension. If the experiment is terminated at a shorter time, uncertainty about the precise point in time at which influx effectively stopped becomes prohibitive. If the study is continued for, say, 20 min, in order to avoid this pitfall, the experiment tends to lose its validity since the influence of the efflux rate constant in the calculation becomes excessive.

The mean value for sodium influx in our experiments was lower than the estimate of sodium efflux derived from the product of the rate constant and the normal value for leucocyte intracellular sodium obtained in our laboratory. There are two possible explanations for this discrepancy. In the first place, the technique described for the estimation of sodium influx may, and probably does, underestimate slightly the true value due to loss of radioactivity from the
sample during the final wash. Secondly, as must be emphasized, the value given for the intracellular sodium content is taken from experiments performed at room temperature. Lacking the knowledge of the $Q_{10}$ for sodium influx and efflux in the leucocyte it cannot be assumed that the values for intracellular sodium will be identical at room temperature and at 37°C.

The relatively small portion of the $^{42}\text{K}$ influx found to be ouabain sensitive is surprising. If all the potassium efflux is assumed to be passive, the passive portion of potassium influx can be estimated if the concentrations of the ion on the two sides of the membrane are known and an estimate of the membrane potential is available. Ussing (1950) showed that the ratio between passive influx and efflux for an ion was a function of the membrane potential and the concentration of the ion in the intracellular and extracellular fluid. Shaw (1955) gives a simple version of the relationship using the ratio of internal to external chloride concentration as an index of membrane potential:

$$\frac{m_o}{m_i} = \frac{c_1}{c_o} \times \frac{[\text{Cl}]_i}{[\text{Cl}]_o}$$

Where $m_o$ and $m_i$ are the passive outward and inward fluxes, $c_1$ and $c_o$ the internal and external potassium concentrations and $[\text{Cl}]_i$ and $[\text{Cl}]_o$ are the chloride internal and external concentrations of chloride. In the leucocyte, the ratio of internal to external chloride concentration has been shown to be approximately 0.5 (Baron & Ahmed, 1969) and we obtained a similar result at room temperature. It can be seen that the ratio of potassium efflux/ouabain-insensitive potassium influx found in the present experiments is very different from that predicted from the Ussing–Shaw equation (approximately 1.5 against a predicted value of approximately 11). This may be taken as evidence that a substantial portion of the ouabain-insensitive potassium influx or of potassium efflux is not the result of free diffusion of this ion down its electrochemical gradient.

It is of interest to compare our results with previous estimates of sodium and potassium transport rates in the leucocyte. Block & Bonting (1964) in the course of an investigation of the adenosine triphosphatase system of leucocytes estimated the sodium and potassium transport rates in chronic myeloid leukaemic leucocytes. This involved measuring the change in intracellular electrolytes resulting from a period of incubation in Tyrode solution in the presence of ouabain. There are several obvious drawbacks to this type of study, the principal one being the changing relationship between intracellular and extracellular electrolyte concentrations during the experiment. Also, the method by which intracellular electrolytes were calculated is not clear from their paper; in particular there seems to have been no attempt to correct the values for extracellular electrolyte trapped in the cell specimens. Finally, it should not be assumed that the flux rates of our normal cells can be compared directly with those of leukaemic leucocytes. It is perhaps not surprising that the values for active transport obtained in the present study are between two and three times those found by Block & Bonting (1964).

Our experiments demonstrate that the leucocyte is a convenient and suitable cell preparation in which to study electrolyte fluxes in man and seems likely to be a useful method for investigating electrolyte disorders in disease states.

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