THE CATION CONTENT OF PHOSPHOLIPIDES FROM SWINE ERYTHROCYTES*

BY LEONARD B. KIRSCHNER

(From the Department of Zoology, The State College of Washington, Pullman, Washington)

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ABSTRACT

Phospholipides from swine erythrocytes were isolated and separated into four reproducible fractions. One of the fractions seems to be pure phosphatidylserine. The others are almost certainly not single compounds, although the analytical data indicate that they represent mixtures considerably simpler than the parent mixture extracted from the cells.

All four fractions contained Na⁺ and K⁺, but very little Ca²⁺. Sodium was the predominant cation in two of the fractions under all conditions although the major intracellular cation was potassium. In the other two fractions the ratio Na/K varied with the extraction procedure largely because the quantity of K⁺ seemed to depend on the solvent system used. There appear to be reasons to believe that the entire system of phospholipides binds Na⁺ preferentially. In addition, it was observed that the quantity of Na⁺ found in the lipide extracts varied when the extrusion of Na⁺ from the cells was made to vary. Both of these observations are consistent with the possibility that the phospholipides play some part in the extrusion of Na⁺ from these cells.

Little is known about the chemical mechanism underlying the active transport of ions. For the past decade nearly all hypothetical schemes have incorporated the concept of a "carrier," an element in the membrane which can form a compound with the ion at some point within the membrane (Steinbach, 1952). Two characteristics of the transport processes can be most satisfactorily integrated within such a scheme; the chemical specificity of the system, and the fact that net movement can occur against an electrochemical gradient that is, in many cases, enormous. While the carrier hypothesis is very generally accepted only a few isolated attempts have been made to identify the components of the pump. Histamine (Erying and Daugherty, 1955) and pyridoxal (Christensen et al., 1954) have been suggested as possibilities, but the evidence for their participation is not strong.

Two recent communications have called attention to the fact that some of the

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phospholipides may play a part in ion movement (Solomon et al., 1956; Kirschner, 1957). Without enumerating here the reasons for choosing the phospholipides as candidates we can outline one requisite of a transport system used to design our experimental approach.

One of the very striking characteristics of ion transport is the chemical specificity of the process. The system responsible for Na⁺ movement across the frog skin will handle only Na⁺ and Li⁺, the latter very badly (Zerahn, 1955) but none of the other alkali metals (Ussing, 1954, 1957). In human erythrocytes even Li⁺ will not substitute for Na⁺ (Maizels, 1954). Thus, in designing experiments to test the possibility that a class of compounds is involved in ion movement two kinds of evidence seem to be critical; a demonstration that the compound can form a “complex” with the free ion, and evidence for specificity in the complex formation.

Early experiments in this laboratory made it apparent that it would be necessary to separate the crude phospholipide extracts into simpler components; not necessarily chemical entities, but fractions which were reproducible and less complex than the mixture derived from the cells. Much of the paper is concerned with a description of the protocols developed in achieving this end, and with a tentative identification of the fractions obtained. More important in the context of this work, data will be presented which are difficult to rationalize except by assuming that at least some of the lipides are, in fact, relatively specific in their binding of sodium.

METHODS

Storage and Incubation of the Erythrocytes.—Swine erythrocytes were used because they maintain a very low intracellular sodium concentration indicating an active extrusion mechanism. Human blood, certainly the best characterized material of this nature, was not available in the quantities used in this work. Many of the early experiments were performed on untreated cold-stored blood. Subsequent work (Kirschner and Harding, 1958) showed that the metabolic properties of the cells as well as their ability to extrude Na⁺ were improved if adenosine was added to the blood during storage. The nucleotide (1 mg./ml.) was added to whole blood immediately after it was brought into the laboratory. Some of the experimental results with adenosine differed from data obtained on untreated blood, and these differences are described and discussed below. In no case was the blood used after it had been stored for longer than 10 days.

Duplicate 150 ml. aliquots of blood¹ were transferred to flasks which were incubated at 39°C. for 4 hours. Mixing was ensured by shaking (80/minute). The blood was then transferred to 250 ml. centrifuge bottles and 100 ml. of warm (45°C.) choline-Ringer's

¹ The duplicates were worked up in parallel through fractionation and analysis. Thus, an experiment such as the one shown in Table V yielded a pair of control and a pair of experimental values. The ion-phosphate ratios in duplicate samples agreed to within ±15 per cent, and replication was usually better than this.
solution was added. After centrifuging at 1000 g for 20 minutes the supernatant fluid was removed by aspiration and discarded. The cells were resuspended and washed twice in warm choline-Ringer's, the final sodium concentration of the extracellular fluid averaging less than 3 mM/liter. At the same time the temperature of the cells probably remained above 30°C. through most of the preparation.

Preparation of the Crude Phospholipide Extract.—After removing the Na-free supernatant from the last washing, each centrifuge bottle was filled with cold (−20°C) acetone, and the packed cells were extracted in the cold for an hour. The suspension was then centrifuged, and the aqueous acetone decanted into a flask. The cell residue was extracted again with acetone, and after centrifugation the supernatant was combined with the first extract. Chloroform-methanol (70:30) was added to the dehydrated residue and it was extracted for an hour. After centrifugation the supernatant was decanted, and the residue extracted twice with petroleum ether. The ether extracts were combined with the chloroform extract.

The acetone extract was taken to dryness in a rotary film evaporator at 15-20°C. Addition of the chloroform-ether extract redissolved the dried material, and the solvent was once more removed. Removal of solvents was carried out in two stages to minimize foaming of the wet lipide at low pressure. Addition of 5 ml. of chloroform-petroleum ether (5:2) dissolved much of the pooled dried material in the flask, and the solution was decanted into a centrifuge tube. In a few experiments the solvent included 22 per cent methanol (cf. discussion of the data in Table III). The insoluble material was centrifuged out, and the clear yellow solution was transferred to another tube. The precipitate was washed with 3 ml. of solvent and the combined supernatants were evaporated to a volume of 1 ml. in a stream of air. Ten volumes of cold (−20°C) acetone were added rapidly, and the tube was placed in the freezer for an hour. The precipitate was packed by centrifugation and the yellow supernatant discarded. The lipide was twice more precipitated from the solvent by acetone; the last supernate was nearly colorless while the precipitate was ivory in color.

Fractionation of the Crude Extract.—One ml. of pyridine was added to the lipide in a centrifuge tube and the suspension stirred intermittently for 15 to 20 minutes. After centrifugation the deep yellow supernatant was transferred to another tube, and the gelatinous residue was washed twice with 0.5 ml. portions of pyridine. The precipitate was stored at −20°C.

Three volumes of cold ethanol were added rapidly to the pyridine solution which was mixed and placed in the freezer for 6 to 10 hours. It was then centrifuged, the precipitate washed twice with ethanol, and the supernatants transferred to a small flask. This precipitate was also stored temporarily in the freezer.

The pyridine-ethanol solution was evaporated to dryness under vacuum, the dried residue taken up in a small volume of chloroform and transferred to a centrifuge tube. Cold acetone was added, and the lipide was packed down by centrifugation. This material was then run through the entire fractionation procedure described above.

Choline-Ringer's is a solution in which Na⁺ is replaced by choline⁺. Its use precluded the possibility of analytical determination of choline⁺ in the lipide hydrolysates. However, the use of isotonic sugar solutions to wash the cells yielded syrupy lipide extracts which were difficult to handle.
A small quantity of pyridine-insoluble material was stored at -20°C. The additional ethanol-insoluble precipitate was also stored. The remaining solution was again evaporated to dryness, and the dry residue stored in the freezer. This material is soluble in both pyridine and ethanol and was labelled fraction 3 (F3).

The pooled ethanol-insoluble lipide was dissolved in pyridine, and any precipitate was centrifuged out and stored. The soluble material was precipitated with 3 volumes of cold ethanol, and after 6 hours at -20°C. it was centrifuged and washed twice with ethanol. The lipide, soluble in pyridine, but insoluble in ethanol was labelled fraction 2 (F2).

The remaining gelatinous precipitates, all insoluble in pyridine, were extracted twice with small portions of pyridine, the solutions being discarded. The residue was then extracted at 5°C. with 5 ml. of wet diethyl ether for an hour. The insoluble material was packed down in the centrifuge, and the ether solution evaporated to dryness under reduced pressure. The ether-soluble lipide was labelled fraction 1E (F1E).

The pyridine-ether insoluble residue was extracted with wet ether, and the ether solution discarded. Though this may entail some loss of residual F1E it should minimize cross-contamination of the two fractions. The ether-insoluble lipide was labelled F1C.

Although four reproducible fractions were produced by this method it is extremely time-consuming and laborious. We have tried chromatography with no success. Silicic acid—impregnated papers (Lea et al., 1955) were used but sodium, which is critical in our work, was pulled off and migrated quite independently of the lipide. Reversed phase chromatography (silicone-vaseline—impregnated paper) gave results which were too variable to be useful, although it was our impression that a systematic survey of this method might prove fruitful.

Analytical Procedures.—Each fraction was dissolved in 2.0 ml. of wet chloroform and diluted to 10.0 ml. with methanol. Sodium, potassium, and calcium were determined directly on these solutions using a Coleman flame photometer. Standards were made up in the same solvent. Combustion of the chloroform caused release of free HCl and it was necessary to perform these analyses in the hood. A few determinations run on aqueous solutions of lipide hydrolysates agreed with the measurements made directly on the lipide.

Lipide phosphorus was determined on perchloric acid digests essentially as described by King (1932). Acyl ester content of the lipide was measured by the hydroxamic acid method adapted for analysis of phospholipide (Entenman, 1957). Amino nitrogen was measured by the ninhydrin method (Moore and Stein, 1948) on solutions which had been hydrolyzed for 40 hours in 6 M HCl (100°C.). The standard used for F1E and F2 was serine prepared by the California Foundation for Biochemical Research. Ethanolamine (from the same source) was used as the standard for F3. Use of these compounds was indicated by chromatographic identification of the ninhydrin-positive material (cf. Table II). An uncharacterized amine was also released during acid hydrolysis of F1C. It is likely that this compound is sphingosine, but since it was not definitely identified no quantitative data were obtained.

Na\(^{2+}\) was obtained commercially in carrier-free solution. Aliquots of the lipide extracts were plated and ashed on stainless steel planchets. The activity of plasma and cellular sodium was determined after plating and drying trichloroacetic acid extracts of hemolysates. Samples were counted with a conventional end-window GM tube.
RESULTS

Hydrolysates of each fraction were spotted on paper strips and the chromatograms were developed with phenol-cyanide. Serine and ethanolamine standards were run at the same time. It can be seen in Table I that the $R_f$ of the ninhydrin-positive compound in F1E and in F2 is the same as that for pure serine. The spot in F3 had the same $R_f$ and migration characteristics (elongated

<table>
<thead>
<tr>
<th>Fraction</th>
<th>No. of spots</th>
<th>$R_f$</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1C</td>
<td>1</td>
<td>0.42</td>
<td>Compact spot</td>
</tr>
<tr>
<td>1E</td>
<td>1</td>
<td>0.38</td>
<td>Compact spot</td>
</tr>
<tr>
<td></td>
<td>compact spot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.59</td>
<td>Elongated spot</td>
</tr>
<tr>
<td>Serine standard</td>
<td>1</td>
<td>0.38</td>
<td>Compact spot</td>
</tr>
<tr>
<td>Ethanolamine standard</td>
<td>1</td>
<td>0.60</td>
<td>Elongated spot</td>
</tr>
</tbody>
</table>

The chromatograms were equilibrated in an atmosphere of phenol-cyanide and then developed with this system. After drying they were sprayed with 0.2 per cent ninhydrin in butanol and heated at 90°C. for 5 minutes.

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>Phosphate</th>
<th>Ester</th>
<th>NH$_2$PO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micromoles</td>
<td>Per cent total</td>
<td>F0$_4$</td>
</tr>
<tr>
<td>1C</td>
<td>13.7</td>
<td>9.4</td>
<td>0.26</td>
</tr>
<tr>
<td>1E</td>
<td>9.9</td>
<td>6.8</td>
<td>1.12</td>
</tr>
<tr>
<td>2</td>
<td>18.1</td>
<td>12.4</td>
<td>1.94</td>
</tr>
<tr>
<td>3</td>
<td>104</td>
<td>71.4</td>
<td>1.81</td>
</tr>
</tbody>
</table>

* Each value is the average of two experiments.
† Ninhydrin analysis on acid hydrolysates.
§ No quantitative data obtained for this fraction (cf. text).

spot) as ethanolamine. The digest from F1C also yielded an amine which moved slightly, but consistently faster than serine. Since the lipide has the solubility characteristics of a sphingolipide, the base is likely sphingosine, and this is consistent with the observation that ninhydrin-positive material from this hydrolysate is soluble in chloroform (cf. McKibbin and Taylor, 1949).

Analytical data for the four fractions are shown in Table II. The values, averages from two representative experiments, have been quite reproducible, although the ester content of F1E is often higher than in these runs. These data, together with the solubility properties of the lipide and the chromatographic evidence described above, permit some tentative estimates to be made
concerning the composition of the fractions. These inferences will be discussed below.

The ionic content of these fractions is shown in Table III. Two sets of data have been tabulated. The experimental procedures were nearly identical, differing only at one stage in the fractionation. In one group the solvent used to redissolve the crude, dried lipide was chloroform:petroleum ether (5:2) as described under Methods. Lipide from the other group was dissolved in chloroform:methanol:petroleum ether (5:2:2). Several facts might be listed here, and discussion reserved until later.

1. Both Na$^+$ and K$^+$ appear in all the fractions.

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Fraction & Solvent 1$^*$ & & Solvent 2$^*$ & & \\
         & Na$^+$/P & K$^+$/P & \&Ca$^{++}$/P & Na$^+$/P & K$^+$/P \\
\hline
1C       & 0.25 $\pm$ 0.14 & 1.40 $\pm$ 1.03 & 0.02 & 0.22 $\pm$ 0.07 & 0.10 $\pm$ 0.02 \\
1E       & 0.39 $\pm$ 0.17 & 1.56 $\pm$ 1.22 & 0.02 & 0.33 $\pm$ 0.11 & 0.10 $\pm$ 0.02 \\
2        & 0.50 $\pm$ 0.10 & 0.31 $\pm$ 0.12 & 0.01 & 0.51 $\pm$ 0.13 & 0.32 $\pm$ 0.09 \\
3        & 0.18 $\pm$ 0.08 & 0.05 $\pm$ 0.04 & 0.01 & 0.18 $\pm$ 0.06 & 0.02 $\pm$ 0.01 \\
\hline
\end{tabular}
\caption{Distribution of Ions in Phospholipide Fractions}
\end{table}

$^*$ The solvent specified was used to dissolve the dried lipide material prior to separation of the phospholipides from other fat-soluble material by acetone fractionation. Solvent 1 was chloroform:petroleum ether (5:2). Solvent 2 was chloroform:petroleum ether:methanol (5:2:2).

$^!$ Each value is an average of data from twenty-two experiments.

$^!$ Each value is an average of data from four experiments.

2. The quantity of sodium in all four fractions is independent of the minor permutation in procedure described above.

3. The quantity of potassium is likewise stable in F2 and in F3 (it is nearly absent from F3).

4. The potassium content of F1C and F1E varies over several orders of magnitude depending on the solvent system used early in the fractionation.

5. In F2 and F3 there is two to three times as much sodium as potassium, and if the high K$^+$ in F1C and F1E is an experimental artifact (as will be argued later) then all the phospholipides contain considerably more sodium.

The last point is of considerable interest, for the cells from which the lipide is extracted contain a large excess of potassium. If the lipide is binding cellular ions it is clearly implied that the process is relatively specific for sodium. The data in Table IV indicate that the lipide is, in fact, deriving its sodium from the cells. At the beginning of a run 10 microcuries of Na$^{25}$ was added to the whole blood. Samples were taken after 2 and 4 hours at 37$^\circ$ and the specific activity of plasma, cellular, and lipide sodium was determined. It can
be seen that the lipide is essentially in equilibrium with cellular sodium, and in another experiment the same results obtained after 6 hours of incubation.

Although it was noted above that the quantity of sodium bound by the lipide is independent of variations in the analytical protocol it is not independent of variations in the state of the cells. Thus, it was reported earlier that the Na/K ratio in F2 and F3 was about 2–3 (Kirschner, 1957). Since that

### TABLE IV

**Source of the Lipide-Bound Sodium**

<table>
<thead>
<tr>
<th>Time at 39°C</th>
<th>Plasma specific activity</th>
<th>Cell specific activity</th>
<th>Lipide specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>124</td>
<td>18.3</td>
<td>20.4</td>
</tr>
<tr>
<td>4</td>
<td>122</td>
<td>35.7</td>
<td>40.2</td>
</tr>
</tbody>
</table>

Whole blood was incubated at 39°C in the presence of 10 microcuries of Na<sup>2</sup>. Specific activity for the Na<sup>+</sup> is in counts per minute per micromole.

### TABLE V

**The Effect of Adenosine on Na<sup>+</sup> and K<sup>+</sup> in the Phospholipide**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>No addition</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na&lt;sup&gt;+&lt;/sup&gt;/P</td>
<td>K&lt;sup&gt;+&lt;/sup&gt;/P</td>
</tr>
<tr>
<td>1C</td>
<td>0.31</td>
<td>0.95</td>
</tr>
<tr>
<td>1E</td>
<td>0.45</td>
<td>2.41</td>
</tr>
<tr>
<td>2</td>
<td>0.51</td>
<td>0.38</td>
</tr>
<tr>
<td>3</td>
<td>0.30</td>
<td>0.05</td>
</tr>
</tbody>
</table>

These experiments entailed use of solvent 1 (Table III), and hence K<sup>+</sup> recovery in F1C and F1E is high. The quantity and composition of the fractions were unchanged by the addition of adenosine.

Note appeared the ratios have been appreciably lower, sometimes little more than unity. The only change in our procedure involved addition of adenosine to the stored blood in the later experiments. Although the averages for sodiums from experiments with adenosine were clearly lower than those from runs without the nucleotide it seemed desirable to compare the ionic ratios in a single experiment. One pair of blood samples was treated with adenosine, the other was untreated; both sets were stored at 5°C for 2 days, then brought to 39°C for 4 hours. Analysis (Table V) showed that lipide from the adenosine-treated cells contained less Na<sup>+</sup> than the controls. Changes in K<sup>+</sup> content of F1C and F1E were not consistent, and the values were essentially unchanged in F2 and F3. It is worth noting that this is the one of the very few experiments in which
the quantity of $K^+$ in F2 exceeded $Na^+$. This is due to the very low content of $Na^+$ in this run; it was, in fact, the lowest seen in a large number of experiments.

**DISCUSSION**

Our primary aim in fractionating the crude phospholipide was to obtain from the parent mixture simpler groups of compounds which would be more amenable to analysis after experimental manipulation. One reason for this procedure is illustrated by the data for solvent 1 in Table III. The $K/P$ ratio for the crude extract would be 0.31; $Na/P$ would be about 0.25. The actual disparity described in the table in values among the four fractions would be missed completely. In addition, the fact that $Na^+$ actually exceeds $K^+$ in fractions 2 and 3 would be masked.

Characterization of the fractions was considered to be the least important part of the work during this phase of the project, and the protocols were not designed toward this end. However, the data presented above allow us to make certain inferences, some supported more strongly than others, about the composition of the fractions. It is nearly certain that F2 is pure phosphatidylserine. The solubility data, and the appearance of serine as the sole amino compound support this conclusion, as does its appearance in 1:1 ratio with phosphate. The ester content is also theoretical for this compound. The lone ninhydrin-positive compound in F3 was ethanolamine, and it corresponded to about 80 to 90 per cent of the phosphate in the fraction. The ester content was consistently below theoretical (ester/P about 1.8), and hence the fraction seems to contain some lysophosphatidylserine.

F1C may be largely sphingolipide. The material is insoluble in pyridine and wet ether; the lipide has a low content of ninhydrin-reactive material, while on hydrolysis a chloroform-soluble amine is liberated; and the ester content is very small. F1E contains sufficient serine to account for all of the lipide. However, its solubility is not that of phosphatidylserine, and the ester values are well below theory for that compound.

However, the main purpose of the work is to explore the possibility that the phospholipides play a part in the movement of ions across cell membranes. It is obvious that for active transport to occur the electrochemical potential of an ion must be raised at one side of the plane of transport. Even our limited knowledge of the molecular structure of cell surfaces allows us to distinguish between a pair of alternative mechanisms for accomplishing this. Since there is a large electrical gradient across many membranes the ion might form a complex with a polycharged molecule so that the net charge of the complex favored diffusion. Thus, $NaX^n$ could move out of a cell or across an epithelium from negative to positive side providing that $n$ is a sufficiently large number.
Such a process could take place in aqueous pores in the membrane as suggested by Ussing (1957).

An alternative mechanism might involve the formation of a complex which is able to "dissolve" in the lipide portion of the membrane. In this case it is the chemical rather than the electrical potential of the complex which underlies diffusion. This possibility has provided the working hypothesis on which this work is based (cf. Kirschner, 1955).

The first step was to look for a constituent of cell membranes which might form a lipide-soluble complex with sodium. It was obvious that at least some of the phospholipides would do this; in fact, their ability to solubilize polar compounds has been noted in the past (cf. Lovern, 1955). Thus, it has been shown that they can carry both sodium and potassium into chloroform-methanol (Folch, 1948; Solomon et al., 1956) and are able to extract ions from aqueous solution into solvents as non-polar as petroleum ether (unpublished experiments).

While this provided evidence that phospholipides might play a role in ion movement, much stronger arguments must be provided to show that they do participate. It has been possible to use the characteristics of the transport process as a guide in designing experiments. Since the active fluxes are chemically specific the lipides were examined for some suggestion of specificity in binding either sodium or potassium. Many of the phosphatides are acidic, and the appearance of cations in the extracts is not surprising. If the extraction is perfectly random, i.e. non-specific, the ratio of cations in the extract should approximate the ratio in the cells. Swine erythrocytes have a large excess of intracellular K⁺, the K/Na ratio ranging from 5–20 depending on the duration of the period of cold storage before they are used. Thus, assuming random extraction of the soluble cations analysis should show that lipide fractions have a large excess of K⁺. This prediction is not borne out by the data in Table III for F2 and F3. For the pyridine-insoluble material (F1C and F1E) the picture is more equivocal. When solvent 1 was used to redissolve the crude lipide K/Na in these fractions approximated the ratio expected for non-specific binding. However, when solvent 2 was used instead, very little K⁺ appeared. There are several reasons for believing that the high K⁺ in these two fractions is an experimental artifact. (1) The quantity of K⁺ found seems to be very sensitive to the solvent used. No other parameter was so affected. The quantity of Na⁺ in this material was the same with both solvents, and this was also true for the composition of the fractions. (2) The quantity of K⁺ varied enormously from experiment to experiment but only in these fractions when solvent 1 was used (cf. standard deviations in Table III). Potassium in the other fractions was stable. (3) Duplicate samples of F1C or F1E frequently showed little agreement; even in the same experiment K values for a pair of duplicates might
differ by a factor of five. This was never encountered in F2 or F3, and it was noted in none of the fractions when the second solvent system was employed. Thus, high potassium sometimes seen in F1C and F1E may be meaningless, and if this is true the entire system of phospholipides contains more Na⁺ than K⁺.

Apparently, then, the cations in F2 and F3, and perhaps in all the phospholipides seem not to satisfy the requirements for simple random extraction from the cells. In an attempt to explain the predominance of Na⁺ several possibilities have been considered. If the lipide is located at the cell surface it might be argued that it is more accessible to the external medium, normally a high sodium solution. This seemed unlikely since the cells were washed with sodium-free solution prior to extraction until the external sodium was reduced to about 2 per cent of its concentration in plasma (at the end of the washing the external K/Na was about 2–3). Further, the data in Table IV show directly that the lipide-bound sodium is in equilibrium with cellular sodium and not with the extracellular ion.

The only alternative seems to be that the lipide can combine with Na⁺ even in the presence of a large excess of K⁺; i.e., the binding is relatively specific for Na⁺. Whether the specificity is inherent in the lipide molecules or in some component (e.g., an enzyme) involved in the turnover of these compounds cannot be decided on the basis of these data. The fact that all four fractions contain an excess of sodium makes it seem less likely that the lipides themselves are specific. It is worth noting, in this context, that phosphatidylserine isolated from ox brain shows K/Na values of 1–3 (Folch, 1948; Lea et al., 1955), although it too is extracted from cells containing a much larger K/Na ratio. If the inference about specificity is correct then the observations would be consistent with a role for phospholipides in sodium extrusion from cells.

Modification of the binding of sodium by inclusion of adenosine in the storage and incubation medium (Table V) was described here primarily to rationalize the difference between the values reported here and those published (for F2 and F3) in our earlier note. However, the observation has a correlate which may prove to be interesting. It has been reported (Kirschner and Harding, 1958) that the use of adenosine increased sodium extrusion by these erythrocytes. Here we see that a change in the ionic pattern of the phospholipides accompanies the more efficient extrusion of sodium from the cell. Obviously, the implications of this apparent correlation are of interest in the context of this work. Work in this direction is under way at present.

**BIBLIOGRAPHY**


LEONARD B. KIRSCHNER

Ussing, H., in Metabolic Aspects of Transport across Cell Membranes, (Q. Murphy, editor), Madison, University of Wisconsin Press, 1957.