STUDIES ON THE DISTRIBUTION OF POTASSIUM IN THE RAT LIVER CELL AND THE MECHANISM OF POTASSIUM ACCUMULATION*

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The importance of potassium as the main cation in plants and in the intracellular portion of most animal tissues has long been recognized. A great number of physiological processes seem to depend on the presence of proper concentrations of potassium. In most instances sodium does not substitute for potassium and often acts as an antagonist. It is, therefore, not surprising that problems connected with the nature and mechanism of potassium accumulation and the role of potassium in biological systems have been and continue to be the basis for many investigations.

The distribution of potassium and sodium in the components of the rat liver cell was studied in order to find out if there is a gradient in K^+ concentration between the soluble and particulate fractions. As far as we know, the only other work on the localization of potassium in specific parts of the cell is that of MACALLUM[1] and, after the completion of our work, a paper on the distribution of potassium and sodium in the rat liver cell has been published by MACFARLANE *et al.*[2]

Studies on potassium retention and accumulation in mitochondria were undertaken because it was found that these particles contain an appreciable amount of potassium, which is apparently needed for their optimal metabolic activity[3]. It was also demonstrated that potassium prevents the loss of respiratory activity in mitochondria[4]. The ease with which these particles are prepared made them particularly suitable for ion studies. In order to preserve the structure of mitochondria as far as possible, all operations were carried out at or near 0° and no additional substrates were added.

EXPERIMENTAL

The liver from male albino rats (Sprague-Dawley strain) was homogenized in a cooled Potter-Elvehjem homogenizer in nine volumes of isotonic sucrose and the homogenate was fractionated in a refrigerated centrifuge according to the SCHNEIDER differential centrifugation technique[5]. The microsomal fraction was obtained in the Spinco Model L Centrifuge at 100,000 × *g* for 30 minutes. For distribution studies, the nuclear and mitochondrial fractions were washed twice with isotonic sucrose and the microsomes were washed once unless stated otherwise. The washings were saved and analyzed for potassium, sodium, and nitrogen in order to obtain balances.

In order to study the effect of different agents and salts on mitochondrial potassium, twice-washed mitochondria representing from 8–10 g liver were diluted with sucrose to 8–10 ml and

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portions were added to lucite tubes containing sucrose and additions. The tubes were incubated in an ice bath for 5–10 min as indicated. The mitochondria were spun down and washed, mostly twice, with isotonic sucrose. They were then analyzed for potassium, in most cases sodium, and for nitrogen in the manner described below.

For potassium accumulation studies, twice-washed mitochondria were incubated in an ice bath for ten minutes with 2,4-dinitrophenol or desoxycholic acid. The mitochondria were spun down, washed once with sucrose and incubated with potassium, ATP* or buffers in an ice bath for 5 minutes. The centrifuging and washing with sucrose were repeated twice more. The time of each spinning was five minutes and the speed approximately 14,000 × *g*. The mitochondria were then diluted to volume with distilled water and the cation and nitrogen determinations carried out. Except for the final dilution in which distilled water was used to facilitate digestion, the suspending medium throughout all the experiments was unbuffered isotonic sucrose.

Aliquots of the total mitochondrial or other fractions were digested with 22–25 *N* sulfuric acid. 6–7 drops of concentrated nitric acid were added to the tubes to facilitate digestion. The digestion was carried out in silica tubes (Vycor, Corning Glass Works). The tubes were cooled and diluted to a volume of 10 ml with freshly boiled distilled water. A potassium and sodium standard and a blank were digested simultaneously with the samples.

The potassium and sodium were determined with a flame photometer attachment on the Beckman DU Spectrophotometer, potassium at a wavelength of 768 mμ, sodium at 589 mμ. Standard curves of light intensity plotted against quantity of alkali cation per unit of volume showed a practically linear relationship. Thus after preliminary calibrations only one K and one Na standard was included in routine determinations. Nitrogen was determined by direct nesslerization.

Potassium and sodium were expressed on the basis of mg/kg liver and on a K^+/N or Na^+/N basis.

ATP was obtained from Pabst Laboratories as the disodium salt and from the Sigma Chemical Company as BaATP. The Pabst ATP contained some potassium and was, therefore, usually purified by converting it to the barium salt and then to the sodium salt. All reagents were neutralized with sodium hydroxide or HCl and contained only traces of potassium.

RESULTS

The data in Table I show the potassium and sodium distribution in rat liver components. The added values for all the components are in good agreement with the potassium and sodium content of the whole homogenate. The potassium and sodium of the homogenate differed only slightly from values found by us for whole liver. Most of the potassium, approx. 75%, is found in the supernatant. The mitochondrial fraction was found to contain slightly less potassium than the nuclear fraction and more potassium than the microsomes. The nuclear fraction contained, in addition to nuclei, connective tissue fragments, a few erythrocytes and unbroken liver cells,

TABLE I

THE DISTRIBUTION OF POTASSIUM AND SODIUM IN RAT LIVER CELL COMPONENTS

	mg K^+/g N	*mg K^+/kg liver*	*mg Na^+/g N*	*mg Na^+/kg liver*
N_w (Nuclei)	34	210	4	34
M_w (Mitochondria)	39	150	3	12
P_w (Microsomes)	16	120	4	38
S_2 (Supernatant)	240	2560	38	390
N washings	95	110	19	23
M washings	120	180	15	20
Whole homogenate	120	3200	20	540

The data are the averages of analyses on 5 livers.

* The following abbreviations are used: DNP = 2,4-dinitrophenol (sodium salt); DCA = desoxycholic acid (sodium salt); ATP = adenosine triphosphate (sodium salt).

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and hence the values obtained may be higher than the actual potassium content of the nuclei. The microsome fraction was washed only once and may have been contaminated with small amounts of soluble potassium salts.

The sodium was likewise concentrated in the supernatant. Of the particulate fraction the nuclei and microsomes contained more sodium than the mitochondria.

In a few experiments the potassium and sodium distribution in liver cell components of alloxan diabetic rats was compared with that of normal ones. It was found in most cases that the total K^+ of diabetic rat livers was lower than that of normal ones and the distribution was somewhat different. However, the data are not included in this paper since the number of experiments was not sufficient to establish the significance of the differences.

Table II shows that a certain amount of potassium can be removed from mitochondria by washing with isotonic sucrose. The amount of potassium leached decreases with successive washings but the K^+/N ratio increases. Since the last two washings contained very little nitrogen, the experimental error involved could be rather large. The potassium in the mitochondria and all three washings represented 14.6% of the total potassium in the homogenate.

TABLE II

K^+ CONTENT OF MITOCHONDRIA AND WASHINGS

	mg K^+/g N	*mg K^+/kg liver*
K^+ in washed M_w	43	103
1st washing	79	113
2nd washing	81	65
3rd washing	100	20

The effect of agents which uncouple oxidation from phosphorylation, *e.g.*, DNP and gramicidin S, on mitochondrial potassium is shown in Table III. Both DNP and gramicidin S caused leaching of mitochondrial potassium and this was not affected by the addition of sodium chloride. However, when sodium ion, which did not influence mitochondrial potassium *per se*, was added in the presence of desoxycholate, the leaching effect was greatly increased. The compounds were neutralized with sodium hydroxide and although a great excess of sodium was present, the mitochondria did not take up significant quantities of this ion. Some variation in the sodium content of the mitochondria could be observed throughout the experiments. The sodium concentration in mitochondria was so low that the considerable variations may have been largely experimental errors. In other experiments (Table V) treatment of mitochondria with 0.013*M* KCl while in the presence of DNP did not prevent loss of K^+ from the mitochondria.

Table IV shows that calcium salts caused potassium leaching while magnesium sulfate had only a slight effect. The addition of sodium salts during treatment with calcium salts had very little effect on the amount of potassium loss produced by calcium.

ATP inhibited the potassium loss caused by DNP as is shown in Table V. The addition of ATP in the presence of DNP and KCl brought the potassium level to almost the same as that of the control to which no DNP was added. There was some

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TABLE III

EFFECT OF SODIUM, UNCOUPLING AGENTS AND SURFACE-ACTIVE AGENTS ON MITOCHONDRIAL POTASSIUM

Treatment	*mg K+/g N**	*mg Na+/g N*	*Treatment*	*mg K+/g N**	*mg Na+/g N*
Control	21	4	Control	23	3
+ NaCl ($1.2 \cdot 10^{-2} M$)	20		+ Desoxycholic acid ($5 \cdot 10^{-5} M$)	11	3
+ DNP ($3.7 \cdot 10^{-5} M$)	13	1	+ DCA ($5 \cdot 10^{-5} M$)		
+ DNP ($3.7 \cdot 10^{-5} M$) + NaCl ($1.2 \cdot 10^{-2} M$)	13		+ NaCl ($1.2 \cdot 10^{-2} M$)	3	3
+ Gramicidin S ($6 \cdot 10^{-5} M$)	3	5	+ Oleic acid ($5 \cdot 10^{-5} M$)	15	2
+ Gramicidin S ($6 \cdot 10^{-5} M$) + NaCl ($1.2 \cdot 10^{-2} M$)	3	5	+ Oleic acid ($5 \cdot 10^{-5} M$) + NaCl ($1.2 \cdot 10^{-2} M$)	12	1

Incubation 5–10 min at 0° C.

* The relatively low K^+ per unit of N may be related to the fact that in these experiments the mitochondria were isolated without washing the nuclear fraction. Furthermore the cell mitochondria used in these experiments were washed twice more than those in Tables I and II. The controls were also washed with sucrose while the experimental samples were washed to free them of DNP or other salts. This applies also to Tables IV and V.

TABLE IV

EFFECT OF CALCIUM AND MAGNESIUM ON MITOCHONDRIAL POTASSIUM

Treatment	*mg K+/g N*	*mg Na+/g N*
Control	32	2
+ $CaCl_2$ ($8 \cdot 10^{-4} M$)	16	1
+ $CaSO_4$ ($8 \cdot 10^{-4} M$)	19	1
+ $MgSO_4$ ($8 \cdot 10^{-4} M$)	26	1

Incubation 10 min at 0° C.

TABLE V

EFFECT OF ATP AND PHOSPHATE BUFFER ON DNP-TREATED MITOCHONDRIA

Treatment	*mg K+/g N*	*mg Na+/g N*
Control	25	3
Treated with $7.5 \cdot 10^{-5} M$ DNP*	15	3
+ $1.3 \cdot 10^{-2} M$ KCl	17	2
+ $5 \cdot 10^{-3} M$ ATP, pH 7.4	19	4
+ KCl and ATP	25	5
Treated with $10^{-4} M$ DNP**	11	
+ $1.3 \cdot 10^{-2} M$ KCl	11	
+ KCl and $5 \cdot 10^{-3} M$ Na-phosphate, pH 7.4	15	
+ KCl and $4 \cdot 10^{-3} M$ ATP	19	

Incubated 5 min at 0°.

* The data are the averages of 4 experiments.

** Average of 7 experiments.

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increase in the sodium retained by mitochondria treated with ATP. This probably resulted from the introduction of large amounts of sodium ion in the ATP solution.

Table V also shows that phosphate buffer permitted the retention of potassium in DNP-treated mitochondria but to a lesser degree than ATP. In other experiments $5 \cdot 10^{-3} M$ glycylglycine at pH 7.4 gave results similar to those with phosphate. Buffers at neighboring pH values from 6.4 to 8.0 were less effective than at pH 7.4.

DISCUSSION

The total rat liver potassium and sodium agree fairly closely with values given in the literature[6,7]. Our K^+/N ratio of approximately 0.1 is identical with the value reported for muscle and other tissues[8]. The potassium and sodium distribution in the liver components is similar to that reported by MACFARLANE *et al.*[2], although the values given by these workers are higher than ours.

In examining the distribution data it has to be realized that we have no evidence that the same distribution holds in intact cells. We did not make any corrections for extracellular electrolytes, and we decided not to perfuse the liver with sucrose before homogenation because of the possibility of introducing further complications. It may well be that the supernatant fraction contains the bulk of potassium even under physiological conditions and that no positive K^+ gradient exists between the particles and the soluble portions of the cell. Reports in the literature indicate that the supernatant contains a large proportion of glycolytic enzymes[9]. Owing to the significance of potassium in glycolysis[10–14], it is logical that the supernatant should contain an abundance of this cation. However, it is clear that mitochondria can maintain a potassium concentration higher than the surrounding medium with the aid of a metabolically available energy source[2,15–18].

In our work a very simple system, uncomplicated by temperature and substrate effects, was chosen for studying potassium accumulation. It was found that low concentrations of uncoupling agents and surface active agents cause leaching of mitochondrial potassium. An examination of normal and DNP-treated mitochondria under the phase microscope (kindly examined by Dr. JOHN HARMAN of the Department of Pathology) revealed that the DNP-treated mitochondria did not differ in appearance from the normal ones. It thus appears that short DNP treatment at 0° does not alter the mitochondrial gross structure and the potassium loss may be connected with an uncoupling effect.

After our work was completed, a paper by SPECTOR[18] appeared in which the effect of various agents on mitochondrial potassium at 0° was studied. The work described here confirms many of SPECTOR's findings.

It is interesting that calcium, long regarded as the physiological antagonist of potassium, caused potassium loss from mitochondria in our experiments. Magnesium, on the other hand, had little effect. Further work will be necessary to determine whether these effects are related to the influence of these ions on ATPase.

The accumulation studies revealed that DNP or DCA-treated mitochondria when incubated with a great excess of KCl took up only a small amount of potassium. When buffers at a pH of 7.4 were included, the K uptake of DNP-treated mitochondria was increased. There was less accumulation of K^+ when more acidic or more alkaline buffers were used. CLELAND[19] has shown that the permeability of heart sarcosomes to KCl is pH-dependent with a minimum at about pH 7.

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Although some variation was found in the sodium content of mitochondria, no conclusive evidence can be drawn that sodium uptake occurred to any appreciable extent. These findings indicate that mitochondria, like intact cells, have a mechanism for excluding sodium.

Recently LING[20] has proposed a new theory of ion accumulation which fits in well with our findings. According to this, potassium accumulation depends on fixed negative charges in the cell. ATP, because of its high adsorption energy, can compete with the fixed protein negative charges for the fixed positive charges. ATP becomes adsorbed on the protein and the fixed negative charges are thus available for association with the cations. The smaller size of the hydrated potassium ion as well as the lower average dielectric constant apparently account for the predominance of potassium over sodium in the cell. It is thus possible that both nonspecific buffers and ATP can play a role in potassium accumulation.

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SUMMARY

The distribution of potassium and sodium in rat liver cell components has been studied. The supernatant fraction contained about 75% of the total liver potassium and sodium. The rest of the potassium was distributed among the nuclei, mitochondria and microsomes. Each of these particulate fractions retained potassium despite repeated washings with 0.25 *M* sucrose or 0.012 *M* NaCl solutions.

Factors influencing the loss and accumulation of mitochondrial potassium have been studied. Uncoupling agents and surface-active agents resulted in loss of potassium from mitochondria. Sodium ion alone had no effect; when added to surface active agents it enhanced the loss of potassium. This did not occur with uncoupling agents. The potassium loss was not compensated by sodium uptake.

Calcium ion caused a loss of potassium. Magnesium had much less effect.

When mitochondria which have been treated with dinitrophenol or desoxycholate were washed free of these agents and incubated with an excess of KCl only a small amount of potassium was taken up. The addition of buffers at pH 7.4 increased potassium accumulation. ATP additions were more effective in that the original potassium concentration in the mitochondria was restored.

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