BBA 3923

RETENTION OF SODIUM AND CHLORIDE BY MITOCHONDRIA

JAMES L. GAMBLE, JR.

Departments of Physiology and Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Md. (U.S.A.)

(Received August 21st, 1962)

SUMMARY

Retention of sodium and chloride by washed mitochondria is described. The observations supplement data reported previously with respect to retention of potassium.

1. Only small amounts of sodium were retained after treatment with electrolyte-free sucrose: 0.025–0.05 μmoles/mg nitrogen or 1.0–2.0 μmoles/ml centrifuged pellet. This sodium was not found to be completely exchangeable with free sodium of the surrounding fluids, and influx of sodium from the medium was not dramatically stimulated by low concentrations (10^{-5} M) of inorganic mercuric salts. These findings differ from those reported with potassium and suggest retention by a different mechanism.

2. Rates of influx of radioactive sodium or potassium into mitochondrial substance were inhibited by high concentrations (10^{-1} M) of other cations in the suspending fluids.

3. Washed mitochondria retained small amounts, also, of chloride: 0.03–0.05 μmole/mg nitrogen. Retention was increased 3–4 fold after treatment with 2,4-dinitrophenol, and this increase was associated with release of equivalent amounts of inorganic phosphate.

INTRODUCTION

Mitochondria retain a relatively stable form of potassium through washing procedures using cold isotonic sucrose[1,2]. This potassium will exchange with free potassium in the surrounding fluids but not with free sodium. On the basis of evidence summarized previously[3], it appears that potassium ions of the inner volume, between the walls, are not restrained through the washing procedure and that ions retained are contained directly in the substance of the wall and cristae.

The experiments of the present report describe absolute quantities and rates of turnover of sodium and chloride of washed mitochondria. It is presumed that these ions also are contained in the membranous substance, but other types of compartmentation may exist.

METHODS AND EXPERIMENTAL PROCEDURE

Mitochondria were isolated, using conventional methods[4], from livers and kidneys of rabbits that had been starved for 24 h. Sucrose, 0.25 M, buffered with Tris, 0.0025 M

(pH 7.4) was used throughout as the suspending medium during homogenization, incubation and washing. As the standard experimental procedure, aliquots of once-washed, freshly prepared mitochondria were incubated for 10 min at 23° in 5 ml of the buffered sucrose. Other reagents were included as described in the legends of the figures. The incubations were terminated by centrifugation at 8000 × *g* for 5 min, and precipitates were resuspended and washed twice prior to analysis. Stable and radioactive sodium and potassium, and mitochondrial nitrogen were determined with use of methods described previously[2]. Mitochondrial chloride was analyzed by the method of COTLOVE[5].

RESULTS

Quantities of potassium, sodium, and chloride retained by washed mitochondria.

The experiment summarized in Fig. 1 was designed to measure the amounts of potassium, sodium, and chloride that remain with the mitochondria through various procedures of incubation and washing. The first study ("control A") gives values obtained when the mitochondria were exposed only to the routine preliminary and final washes. Next, for "control B," a 10-min incubation in isotonic sucrose was interposed. The somewhat lower column for potassium indicates a loss during treatment with a potassium-free solution at room temperature. In the next vessel the mitochondria were incubated in 0.1 M KCl, and no decrease in the content of potassium was observed. This suggests increased stability or some compensatory absorption of potassium from the medium. When 0.1 M KCl was replaced by 0.1 M NaCl, the drop in potassium was observed again as in "control B." The amount of sodium retained in this study remained small; but a measurable increase to approximately twice the control values was observed.

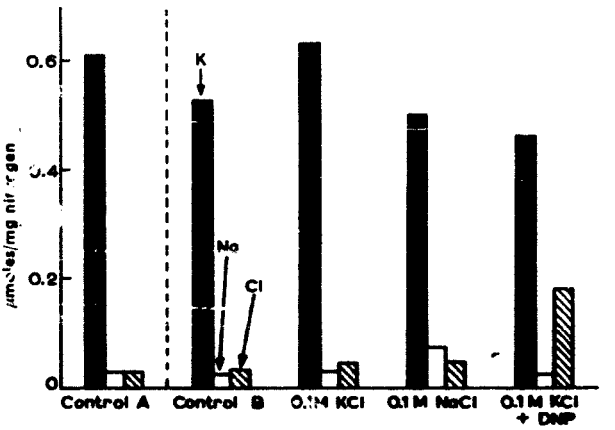

Fig. 1. Total amounts of potassium, sodium and chloride retained by washed liver mitochondria. Equal aliquots from a single preparation were incubated for 10 min at 23° in 5 ml of 0.25 M sucrose, buffered with Tris, 0.0025 M (pH 7.4). This buffered sucrose was used throughout as the medium of suspension for incubation and washing. In all instances the freshly prepared, once washed mitochondria were exposed to two final washing procedures. "Control A," no incubation period included; "control B," aliquot incubated in the buffered sucrose without added sodium or potassium. Additional reagents were included during the incubation periods in the companion experiments as indicated. DNP designates 2,4-dinitrophenol $2 \cdot 10^{-5}$ M.

In the final experiment, the mitochondria were incubated in the presence of 2,4-dinitrophenol at $2 \cdot 10^{-5}$ M, a concentration sufficient for complete inhibition of oxidative phosphorylation. Here the potassium column is significantly lower in comparison to the measurement made in 0.1 M KCl without dinitrophenol; but the more dramatic effect is the increase in the content of chloride. This aspect is considered in more detail with discussion of the data of Fig. 5.

Rates of influx of sodium and potassium

The data of Fig. 2 consider the rates at which exogenous potassium and sodium ions are incorporated into the mitochondrial substance to become "bound" in such a way that they are not removed by subsequent washing procedures with isotonic sucrose. Mitochondria from rabbit kidney were exposed to 0.005 M and 0.1 M KCl labeled with ^{42}K (upper curves) or 0.005 M and 0.1 M NaCl labeled with ^{22}Na (lower curves). The solid lines designate the total amounts retained and the dotted lines the influx from the medium. Influx was calculated by dividing the radioactivity of the washed mitochondria by the specific activity of the electrolyte of the incubation medium.

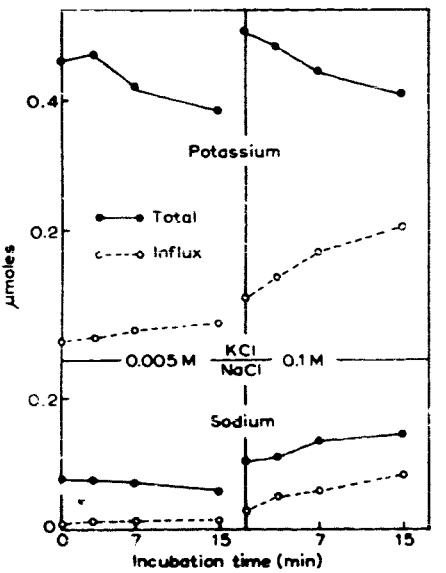

Fig. 2. Total amounts of potassium or sodium retained after various times of incubation. The dotted lines designate the influx as calculated from measurements of the radioactivity of the washed mitochondria. Equal aliquots of kidney mitochondria (1.3 mg nitrogen) were incubated in 5 ml of the buffered sucrose containing 0.005 M or 0.1 M KCl labeled with 0.4 μC of ^{42}K and simultaneously in 0.005 or 0.1 M NaCl labeled with 2 μC of ^{22}Na. The mitochondria were washed twice after incubation.

Influx of potassium was achieved without increase in the total amount retained which is indicative of exchange activity (see ref. 2.). On the other hand, influx of sodium when the medium contained 0.1 M NaCl, was associated with an increase in the total. In this case it appears as though most of the newly acquired material was simply added to the original. The presence of some exchange activity, however, is not ex-

cluded. The data for both sodium and potassium demonstrate a larger influx with exposure to higher concentrations of electrolyte.

Comparative effects of mercury upon influx of sodium and potassium.

The study of Fig. 3 presents a side-by-side comparison of the effect of mercury upon rates of influx of potassium and of sodium. Aliquots of liver mitochondria from a single preparation were exposed to 0.005 M KCl labeled with ^{42}K and separately to 0.005 M NaCl labeled with ^{22}Na. The mitochondria were washed twice prior to analysis.

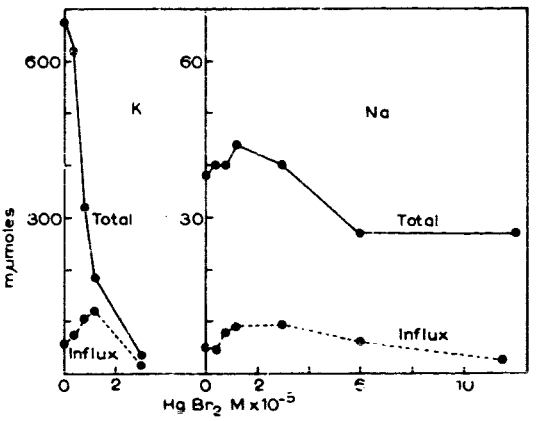

Fig. 3. Effects of increasing concentrations of $HgBr_2$ upon the binding of potassium and sodium. The solid lines refer to the total amounts of electrolyte retained after washing and the dotted lines to the influx as calculated from measurements of the retained radioactivity. Equal aliquots of liver mitochondria (0.9 mg nitrogen) were incubated for 10 min in 0.005 M KCl labeled with ^{42}K and simultaneously in 0.005 M NaCl labeled with ^{22}Na. Mitochondria were washed twice prior to analysis.

As reported previously[6], exposure to mercury, 10^{-5} M, for 10 min induces release of potassium and, simultaneously, a 4- to 6-fold increase in the rate of exchange or of turnover of the material remaining. Increased rate of exchange serves to explain the increased influx observed when the concentration of mercury was 10^{-5} M. At higher levels the total amount retained was reduced greatly, and despite a high specific activity, approaching that of the media, only small amounts of the labeled material were retained.

Comparable observations of the effect of mercury upon sodium revealed two important differences. First, the capacity to retain sodium was not abolished at the higher concentrations of mercury; and second, the calculated influx did not exceed 20–30 % of the total.

Inhibition of the rate of potassium influx with addition of other cations

Incubation of mitochondria with labeled potassium results in an incorporation and retention of radioactivity as a manifestation of the potassium exchange reaction[3]. Earlier studies[3] have shown that the rate of influx by this mechanism is inhibited in the presence of increasing concentrations of sodium. The data of Fig. 4 compare inhibitory effects of Na^+, with those of NH_4^+, and lysine. Lysine, with 2 NH_3^+ groups and one $—COO^-$, was inhibitory, whereas glycine, with one of each, was not. K^+,

NH_4^+ and lysine have been observed to induce qualitatively similar inhibition of the influx of labeled sodium.

Inhibition of potassium influx by sodium is not due to replacement of one ion for the other. The amount of retained sodium is insufficient to account for the results in this way. In one experiment with 0.04 M KCl in the medium, raising the concentration of NaCl, from zero to 0.1 M, reduced influx of potassium from 203 to 110 mμmoles in a 10-min period. Retained sodium was increased, but only from 22 to 39 mμmoles.

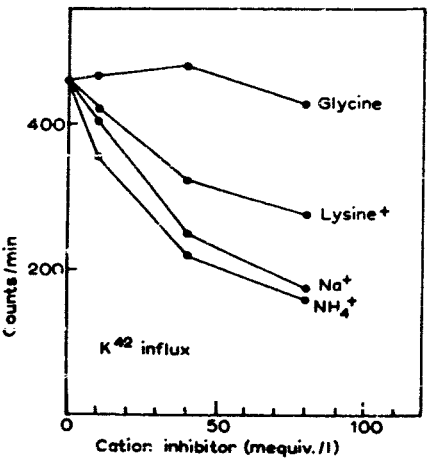

Fig. 4. Inhibition of potassium influx with increasing concentrations of other cations. Equal aliquots of liver mitochondria (1.8 mg nitrogen) were incubated for 10 min in 0.01 M KCl labeled with ^{42}K in the presence of increasing concentrations of glycine, lysine·HCl, NaCl and NH_4Cl. The particles were washed twice prior to analysis.

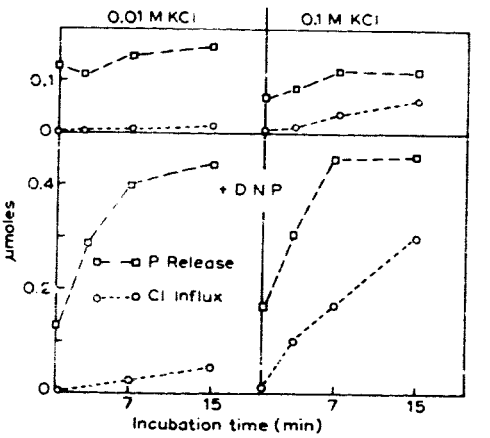

Fig. 5. Effect of dinitrophenol upon the release of inorganic phosphate and upon the influx of labeled chloride from the media. The influx was calculated from measurements of the radioactivity of the finally washed mitochondria. Equal aliquots of liver mitochondria (1.7 mg nitrogen) were exposed to 0.01 or 0.1 M KCl labeled with ^{36}Cl. The figure describes results obtained in the absence (upper curves) and in the presence of $2 \cdot 10^{-5}$ M 2,4-dinitrophenol.

Chloride uptake in the presence and absence of dinitrophenol

As was the case with sodium, only small amounts of chloride were retained by washed mitochondria. A small influx of radioactive chloride was measurable but again, certain distinction between net uptake and influx due to exchange was not achieved. Studies with radioactive ^{36}Cl and ^{82}Br have shown an accelerated rate of influx with exposure to 2,4-dinitrophenol. This effect is shown in the experiment of Fig. 5 to be related to phosphate metabolism. The data compare amounts of exogenous labeled chloride retained with amounts of endogenous phosphate released after exposure to the inhibitor. The original, chemically determined content of chloride of these samples was 0.06 μmole. In the absence of 2,4-dinitrophenol the calculated influx of labeled chloride was 0.015 or 0.06 μmole when exposed to 0.01 or 0.1 M KCl, respectively. These increased to 0.05 and 0.30 μmole, respectively, with addition of dinitrophenol. The amount of phosphate released was increased from approx. 0.1–0.4 μmole with addition of the inhibitor. Change in the concentrations of KCl did not affect the release of phosphate. Qualitatively similar results have been obtained in studies with labeled

bromide. Increased retention of the halides is a relatively unstable property, one that is lost with aging or with repetitive washing.

DISCUSSION

The data of Figs. 2 and 5 describe progressive increments in the radioactivity retained by the washed mitochondria. If, as suggested in the introduction, the ions are retained directly in the wall and cristae, these data measure influx into the membranous substance. This interpretation is supported by the earlier findings with potassium showing rates of influx into isolated fragments of the membrane that are similar to rates recorded for the washed, whole mitochondria[2]. The data of Fig. 4 suggests competition between the cations for channels of entry.

Relatively small amounts of sodium were retained by the washed particles (Fig. 1). In addition, studies described in Figs. 2 and 3 have indicated that a portion of the sodium is not readily exchangeable, and that sodium is only incompletely removed with exposure to mercury. These data differ from those reported for potassium[2,3]. They suggest that a portion of the sodium is present in a relatively inert form. Incubation with the mercuric salts, however, did produce a moderate increase in influx (Fig. 3); and this partial simulation of results obtained with potassium may mean that up to a fourth of the sodium is held by the same forces that control potassium. It is noted, as an indication of the specificity of the potassium retention mechanism, that 1/4th of the retained sodium amounts only to 1/80th of the retained potassium.

Two- to threefold increments in the content of chloride were demonstrated after incubation with 2,4-dinitrophenol. This effect is related to the dinitrophenol stimulation of inorganic phosphate, a phenomenon described by GREEN *et al.*[7]. Presumably labilization and release of phosphate creates a positively charged acceptor site for retention of chloride. The relationship, however, was not found to be an obligate one since reduction in the concentration of KCl in the medium reduced chloride influx without affecting phosphate release.

Dinitrophenol produced qualitatively similar results in studies with labeled bromide. In the mitochondria, as in the intact cell, similarities in the distribution of halides contrast with dissimilarities observed with Na^+ and K^+.

ACKNOWLEDGEMENTS

This investigation was supported by Research Grant Number A-1775 from The National Institutes of Health, Bethesda, Md. This work was done during the tenure of an Established Investigatorship of the American Heart Association by the author.

REFERENCES

1 S. W. STANBURY AND G. H. MUDGE, *Proc. Soc. Exp. Biol. Med.*, 82 (1953) 675.
2 J. L. GAMBLE, JR., *J. Biol. Chem.*, 228 (1957) 955.
3 J. L. GAMBLE, JR., *Am. J. Physiol.*, 203 (1962) 886.
4 V. R. POTTER, in W. W. UMBREIT, R. H. BURRIS AND J. F. STAUFFER, *Manometric Techniques*, Burgess Minneapolis, 1957, p. 180.
5 E. COTLOVE, *Biochem. J.*, 82 (1962) 22 P.
6 R. L. SCOTT AND J. L. GAMBLE, JR., *J. Biol. Chem.*, 236 (1961) 570.
7 D. E. GREEN, W. A. ATCHLEY, J. NORDMANN AND L. J. TEPLY, *Arch. Biochem.*, 24 (1949) 359.