8. The reduction of coenzymes by malate and succinate was much less sensitive to dinitrophenol than that by pyruvate; relatively high ratios (2.5–18) of reduced to oxidized coenzyme could be obtained with concentrations of dinitrophenol of 10 μ M and 0.1 mM.

9. The maintenance of reduced coenzyme by endogenous substrate and pyruvate was enhanced for a short time by amytal, the effect usually being maximal after about 30 min. of incubation. Subsequently there was a rapid decline in the amount of reduced coenzyme.

10. On the basis of the results obtained, it is proposed that the function of mitochondrial reduced triphosphopyridine nucleotide is that of a reserve of reducing power, which is supplemented when the oxidation of substrate exceeds the capacity of the electron-transport chain and which is utilized in the preservation of the level of reduced diphosphopyridine nucleotide when there is little oxidizable substrate. The significance of the data obtained with dinitrophenol for the formulation of the mechanism of the reduction of mitochondrial coenzymes by succinate and the evidence obtained for 'compartmentation' of these coenzymes is also discussed.

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Exchange of Potassium Ions across a Concentration Difference by Isolated Rat-Liver Mitochondria

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Several experiments have been described which suggest that isolated mitochondria are capable of active transport (Spector, 1953; Macfarlane & Spencer, 1953; Stanbury & Mudge, 1953; Bartley & Davies, 1954; Mudge, 1955; Price, Fonnesu & Davies, 1956; Gamble, 1957; Bartley & Amoore, 1958). Nevertheless, it is difficult to prove that a given tissue can perform active transport. Rosenberg (1954) emphasized the necessity of showing that transport of solute from lower to higher electrochemical potential had occurred. The

electrochemical potential should include terms representing both the chemical-concentration difference, and the electrical-potential difference, existing across the membrane (Ussing, 1949). Nothing seems to be known about the potential across the mitochondrial membrane, so the present argument is limited to consideration of the chemical-concentration differences. Even within these restricted terms of reference, however, the above-mentioned experiments are not entirely convincing, because of the following considerations. Spector (1953), Stanbury & Mudge (1953), Mudge (1955) and Gamble (1957) measured move-

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ments of K^+ ions with respect to the nitrogen or dry-matter content of the mitochondria, without measuring the water content of the particles. They did not show that a concentration difference of K^+ ions existed between particles and medium during the incubation. The isolated mitochondria used by Macfarlane & Spencer (1953) and by Price *et al.* (1956) may have been considerably swollen, in view of later work by Werkheiser & Bartley (1957), and it is possible that the apparent movements of ions observed were largely due to changes in the fluid content of the mitochondria. In the experiments of Bartley & Davies (1954) the exchanges of Na^+ and K^+ ions between particles and medium proceeded so rapidly that it is doubtful whether an effective barrier to free diffusion of these ions was present; furthermore, their results on uptake of citric acid-cycle intermediates and of phosphate may have been influenced by adsorption, or by hydrolysis of labile phosphates during centrifuging (Amoore, 1958). Although some of the uptake of Mn^{2+} ions by mitochondria seemed to be an active process (Bartley & Amoore, 1958), these ions are strongly bound by the mitochondrial surface, so the concentration of free Mn^{2+} ions within the particles may have been no greater than that in the medium.

This paper describes experiments in which an attempt has been made to meet such criticisms.

METHODS

The general experimental procedure and analytical methods were as previously described (Werkheiser & Bartley, 1957; Amoore & Bartley, 1958). Rat-liver mitochondria were prepared by homogenizing and differentially centrifuging in 0.25 M-sucrose at 0°. At each stage of the experiment, the mitochondria in samples (2 ml.) of the suspension were centrifuged through 2.5 ml. of silicone fluid of sp.gr. 1.050. (No lower layer of aqueous 'fixative' was used.) When the centrifuge stopped, the tubes were placed in ethanol-solid CO_2 to freeze the contents. By this procedure, the mitochondrial pellets were promptly separated from the suspending medium, and no further redistribution of ions could occur. The mitochondrial pellets and supernatant fluid were recovered, extracted with 10% (w/v) trichloroacetic acid and the extract was analysed (Werkheiser & Bartley, 1957).

Extraction of lipid materials. Instead of being extracted with 10% trichloroacetic acid, two of the mitochondrial pellets were subjected to the following procedure for extracting lipids. This procedure was based on that of Solomon, Lionetti & Curran (1956), with modifications by Mr R. Hems of this Laboratory. The pellets, without being allowed to thaw, were 'freeze-dried' over P_2O_5 and extracted three times with $CHCl_3$ -methanol (2:1, v/v) at 60°. The combined extracts were evaporated to dryness and the residue was extracted three times with $CHCl_3$ (previously dried over $CaCl_2$). The $CHCl_3$ extracts were combined and diluted to 10 ml. with $CHCl_3$ for determinations of radioactivity in a liquid counter. Then they were evaporated to dryness again, the $CHCl_3$ -soluble residues were ashed with

conc. HNO_3 and diluted for potassium estimations by flame photometry. The $CHCl_3$ -insoluble residues were also ashed with conc. HNO_3 and assayed for radioactivity and for potassium.

Radioactivity determinations. The suspensions of mitochondria contained both [^{14}C]carboxypolyglucose (to measure extraparticulate fluid) and ^{42}KCl (to measure exchange of K^+ ions). The ^{42}K was measured with a liquid counter. The ^{14}C was measured with an end-window counter, after sufficient time had elapsed for the counting rate due to ^{42}K to have decreased to less than 1% of that due to ^{14}C . The water contents of both the mitochondria alone and of the whole mitochondrial pellet were calculated. The estimations of mitochondrial water were less consistent than were the estimations of total pellet water. This was probably because the estimations of extraparticulate fluid by means of [^{14}C]carboxypolyglucose were made without the refinements later found to be desirable (Amoore, 1958). Accordingly the concentrations of solutes in the water of the whole pellet were calculated and are shown in Table 1.

Measurements of pH. These were made with a Beckman pH meter model G, with the probe-assembly type 14400. The glass electrode was standardized with a phthalate buffer. The probe was inserted into the centrifuge tubes to measure the pH of the mitochondrial suspension before centrifuging. The buffering capacity of suspensions of mitochondria was estimated by titrating with N-HCl and measuring the pH with the glass electrode. The acid was delivered at a rate of 1 μ l./min. from an Agla micrometer syringe (Burroughs Wellcome and Co.), which was driven by a synchronous motor (Smith's Setric light-duty constant-speed motor). This arrangement is due to Mr A. Renshaw of this Laboratory (unpublished design). The mitochondrial suspension (2 ml.) was contained in a glass centrifuge tube placed in an ice bath, and was stirred by a gentle stream of CO_2 -free air. The pH of the suspension was read at noted time intervals after starting the motor.

Definitions. In this work, the distributions of solutes were considered in terms of three different water spaces. Generally, the 'total water content of the pellet' was employed, as in Table 1. The 'internal water content of the mitochondria' represents the water volume which was not penetrated by [^{14}C]carboxypolyglucose. The 'inaccessible space of the mitochondria' represents the water volume which was not penetrated by sucrose. These quantities were calculated as described by Werkheiser & Bartley (1957) and by Amoore & Bartley (1958). Reasons have been given (Amoore & Bartley, 1958) for considering that only the 'inaccessible space' is surrounded by a barrier to free diffusion of sucrose and Na^+ , K^+ and Cl^- ions.

EXPERIMENTAL AND RESULTS

In an experiment described previously (Amoore & Bartley, 1958) it was shown that when rat-liver mitochondria were re-suspended in ice-cold 0.125 M-potassium chloride containing ^{42}K , the endogenous potassium of the mitochondria exchanged only very slowly with the labelled K^+ ions of the medium. In 15 min. practically no exchange occurred; in 1 hr. less than one-tenth of the endogenous potassium exchanged, and even after 8 hr.

only three-quarters had exchanged. Mudge (1955) has shown that the rate of exchange of the endogenous potassium of rabbit-liver mitochondria with ^{42}K in the medium was about three times as fast at 25° as at 1° . In the following experiment a suspension of rat-liver mitochondria was incubated at 21° .

A medium composed of 0.04M-potassium chloride and 0.25M-sucrose was employed. A preliminary experiment showed that in this medium a concentration difference between mitochondria and medium of about 60 m-molal potassium was obtained, and that during 15 min. at 0° no exchange of mitochondrial potassium occurred.

Distribution of solutes at 0° . Rat-liver mitochondria were isolated in 0.25M-sucrose and [^{14}C]-carboxypolyglucose was added. A sample of the suspension was centrifuged for analysis of the supernatant fluid and the mitochondrial pellet. The pellets had a water content of 2.74 l./kg. of *M*, where *M* represents mitochondrial dry weight (Table 1). The pellets contained 38.2 m-molal potassium and 5.6 m-molal chloride. Potassium chloride (1.5M) was added to the suspension so as

to produce a final concentration of about 0.04M-potassium chloride. From here until the end of the experiment the mean concentration of sucrose in the supernatant medium was 245 m-molal, and the mean concentration of potassium chloride was 42.0 m-molal. After allowing 20 min. for equilibration, the mitochondrial pellets obtained had a decreased water content of 2.48 l./kg. of *M*, and increased concentrations of potassium and chloride (74.7 and 34.7 m-molal respectively). The excess of concentration of potassium, which is the difference between the concentrations of potassium in pellet and medium, was only a little less than it was before potassium chloride was added to the medium. The concentrations of both sucrose and chloride were substantially less than those present in the medium.

At time 24 min., a trace of ^{42}KCl was added to the suspension, and samples were immediately centrifuged. From the specific activity of the potassium in the supernatant fluid and in the mitochondrial pellet it was concluded that the labelled potassium reached a concentration of only 36.1 m-molal in the pellet. The difference between this and the total concentration of potassium in the pellet

Table 1. *Effect of incubation at 21° on the exchange of mitochondrial potassium with ^{42}K in the medium*

A suspension of rat-liver mitochondria in 0.25M-sucrose (34 ml., containing 0.5% of [^{14}C]carboxypolyglucose) was kept at 0° in a stoppered 50 ml. measuring cylinder. At time 0 min., two samples (2 ml.) were withdrawn and centrifuged at 0° for analysis of the mitochondrial pellet and supernatant fluid. At time 1 min., 0.80 ml. of 1.5M-KCl was added to the remaining 30 ml. of suspension, and at time 20 min. two more samples were centrifuged at 0° . At time 24 min., 2.6 ml. of 0.04M- ^{42}KCl in 0.25M-sucrose was added to the remaining 26 ml. of suspension, and 1 min. later three samples were centrifuged at 0° . (The pellet from one of these samples was preserved frozen for extraction of lipids.) At time 29 min., the remaining 22 ml. of suspension was poured into a 150 ml. conical flask (open to the air) placed in a Dubnoff metabolic shaking incubator (Precision Scientific Co., Chicago, Ill., U.S.A.) containing water at 21° . Two samples were immediately withdrawn and centrifuged at room temperature. Single samples were subsequently withdrawn and centrifuged (at room temperature) at times 35, 40, 45, 50 and 70 min. Finally, at time 90 min., two samples were centrifuged and the pellet from one of these samples was preserved frozen for extraction of lipids. The table shows means when duplicate pellets were analysed. Throughout the experiment, the mean concentration of sucrose in the supernatant medium was 245 m-molal, and (apart from the first sample) the mean concn. of KCl was 42.0 m-molal. The 'excess' of potassium in the pellet represents the difference between the total concentration of potassium in the pellet and that in the medium. The 'unexchanged' potassium in the pellet represents the difference between the total concentration of potassium and the concentration of ^{42}K -labelled potassium in the pellet. *M* represents mitochondrial dry wt.

Time (min.)	Temp.	Internal water content of mitochondria (l./kg. of <i>M</i>)	Total water content of pellet (l./kg. of <i>M</i>)	Concn. of solutes in pellet (m-molal)					
				Sucrose	Chloride	Potassium			
						Total	Excess	^{42}K	Unexchanged
0	0°	1.71	2.74	178	5.6	38.2	37.8	—	—
1		0.04M-KCl added to suspension							
20		1.34	2.48	183	34.7	74.7	32.7	—	—
24		^{42}KCl added to suspension							
25		1.45	2.57	185	35.6	76.4	34.4	36.1	40.3
29	21°	Suspension warmed to 21°							
30		1.10	2.59	185	34.2	77.4	35.4	38.0	39.4
35		1.44	2.58	188	35.5	78.4	36.4	39.3	39.1
40		1.34	2.55	196	36.1	79.9	37.9	45.8	34.1
45		1.36	2.61	193	35.8	78.6	36.6	45.8	32.8
50		1.50	2.68	198	37.5	78.0	36.0	48.0	30.0
70		1.73	3.05	212	37.9	67.3	25.3	52.2	15.1
90		2.35	3.91	236	43.0	52.8	10.8	50.2	2.6

was 40.3 m-molal. This unexchanged potassium corresponded exactly with the endogenous potassium concentration of the mitochondria, after allowing for the shrinkage produced by the increased concentration of the medium. The above measurements of the distribution of solutes at 0° were similar to previous findings (Amoore & Bartley, 1958).

Effect of incubation at 21° on the distribution of solutes. At time 29 min. the remaining suspension was rapidly warmed to 21°, and incubated at this temperature for 1 hr. Samples were withdrawn and centrifuged at times 30, 35, 40, 45, 50, 70 and 90 min. from the start of the experiment. The total water content of the mitochondrial pellets was approximately constant at about 2.6 l./kg. of M during the first 20 min. of incubation at 21° (until time 50 min.), but thereafter the mitochondria swelled until at time 90 min. the water content of the pellet was 3.9 l./kg. of M , an increase of 50%. The concentrations of sucrose and chloride in the pellets did not alter during the first 20 min. of incubation at 21°. However, accompanying the swelling of the mitochondria, there were increases in the concentrations of sucrose and chloride, until at time 90 min. both solutes had nearly the same concentrations in the pellet as in the supernatant fluid. Thus the mitochondria became completely penetrated by sucrose and chloride in 1 hr. at 21°, whereas during 8 hr. at 0° a substantial proportion of the mitochondrial volume had remained inaccessible to these solutes (Amoore & Bartley, 1958).

For the first 20 min. of incubation at 21° the total concentration of potassium in the mitochondrial pellets remained constant, but it then decreased until at time 90 min. it was only 10.8 m-molal greater than that in the medium. Thus at the same time that the above-mentioned changes in water, sucrose and chloride content took place, the mitochondria lost their ability to retain a higher concentration of potassium than that in the medium. The only change in the mitochondria observed during the first 20 min. of incubation at 21° was an increase in the radioactivity of the pellets. During the whole period of the incubation the concentration of unexchanged potassium in the mitochondrial pellets steadily decreased, until at time 90 min. only 2.6 m-molal potassium remained unexchanged.

The internal potassium of the mitochondria was diluted by the increase in water content which occurred. Therefore the results were also calculated in an alternative manner on the basis of dry weight, and expressed as solute contents (m-moles/kg. of M). The changes in excess of potassium and unexchanged potassium are shown graphically in Fig. 1. This confirms the conclusion that during the

first 20 min. of incubation at 21° the mitochondria were able to maintain a constant concentration of potassium above that in the medium, while part of that potassium was undergoing exchange with that in the medium. During this 20 min., 22% of the endogenous potassium content of the mitochondria exchanged with that in the medium, and at the end of an hour, 91% had exchanged.

Extraction of lipid-bound potassium. It was considered possible that the endogenous potassium in the mitochondria might be bound by lipid materials, and that this might account for its failure to exchange at all rapidly with potassium in the aqueous medium. If that were so, the endogenous potassium should be extractable from the mitochondria with solvents for lipids. To test this, at times 25 and 90 min. (Table 1) extra samples of the mitochondrial suspension were centrifuged, the mitochondrial pellets were 'freeze-dried', and their lipid constituents were extracted. It was found that only 2.2% of the potassium in the mitochondrial pellets was soluble in chloroform. This was far too small to account for the proportion of potassium in the pellet (53%) which was initially unexchangeable at 0°. Indeed, the 2.2% might represent the minute quantity of potassium extracted from its inorganic salts by chloroform under these conditions (Solomon, Lionetti & Curran, 1956). Of the total radioactivity in the mitochondrial pellets, 1.6% was soluble in chloroform at time 25 min. and 2.5% at 90 min.

Adsorption of potassium and magnesium by mitochondria. Although little of the mitochondrial

Fig. 1. Changes in potassium content of mitochondria during incubation at 21°. The data on the distribution of potassium were taken from Table 1 and recalculated on the basis of mitochondrial dry wt. (M), instead of water content of the pellet. ●, Potassium content of the mitochondria in excess of the concentration in the medium; ○, potassium content of the mitochondria which remained unexchanged with ^{42}K in the medium.

potassium was lipid-bound, some potassium may be bound in other ways. Amore & Bartley (1958) showed that when potassium chloride was added to mitochondria suspended in 0.25 M-sucrose, there was a greater increase in the potassium content than in the chloride content of the mitochondria. In the experiment shown in Table 1, the difference was 11 m-moles of potassium/kg. of *M*. The following experiment suggests that this extra potassium was probably adsorbed by the mitochondria in exchange for other ions, especially H^+ , in much the same way as Mn^{2+} ions are taken up (Bartley & Amore, 1958).

The pH of a sample (2 ml.) of mitochondrial suspension in 0.25 M-sucrose at 0° was 7.17. When 0.16 ml. of 0.125 M-potassium chloride was added (which gave a concentration of 10 mM-potassium chloride in the medium) the pH decreased to 7.10. The sample was then centrifuged for analysis. Titration of a fresh sample with hydrochloric acid (see the Methods section) showed that the mitochondrial suspension had a large buffering capacity. Therefore the above small change of only 0.07 pH unit represented the production of a considerable amount of acid. It was found by analysis that the difference between the increases in potassium and chloride concentrations of the mitochondria was 7 m-moles of potassium/kg. of *M*. About two-thirds of the uptake of potassium by the mitochondria was compensated by release of H^+ ions, and the remainder by release of Mg^{2+} ions.

When magnesium chloride (final concentration 5 mM) was added to a further sample of the same suspension of mitochondria in 0.25 M-sucrose the fall in pH was 0.26 unit. The quantity of magnesium adsorbed by the mitochondria (in excess of chloride) was 26 m-equiv./kg. of *M*. Again most of the uptake was compensated by release of H^+ ions, but there was also a small loss of K^+ ions from the mitochondria.

DISCUSSION

Concentration difference of potassium. The data in Table 1 show that throughout the experiment the concentration of potassium in the mitochondrial pellet was greater than that in the medium. However, the true concentration difference was probably greater than that indicated in Table 1, because the endogenous potassium of the mitochondria was probably restricted to that phase of the mitochondrial water which was inaccessible to sucrose (Amore & Bartley, 1958). On this assumption, the concentration of potassium within this inaccessible space was calculated to be 137 m-molal. When this calculation was applied to several different preparations of mitochondria, it showed that the concentration of potassium in the non-accessible water was in the range 101–158 m-molal.

The probable concentration of potassium within mitochondria is similar to that believed to exist in the interior of liver cells (Aebi, 1953). Hence there may be no concentration difference of potassium between liver mitochondria and their surroundings *in vivo*, and therefore no requirement for active transport of potassium.

Some of the endogenous potassium may be bound by adsorption or associated with lipids. The amount extracted with a solvent for lipids was found to be less than 5% of the endogenous potassium. In the presence of 42.0 m-molal potassium chloride, the mitochondria took up potassium in excess of chloride. The amount so taken up (probably by adsorption in exchange for H^+ and Mg^{2+} ions) was equivalent to only about 10% of the quantity of endogenous potassium. It was not possible to estimate how much of the endogenous potassium was bound to adsorption sites within the mitochondria, but competition with endogenous Mg^{2+} ions would tend to decrease the binding of endogenous potassium. However, indirect evidence that most of the endogenous potassium of mitochondria is in free solution is provided by the fact that the non-accessible volume of isolated mitochondria shows an osmotic responsiveness (Amore & Bartley, 1958). Analysis has shown no endogenous solutes other than the potassium phosphates which could account quantitatively for the internal osmotic pressure of the mitochondria. Gamble (1957) suggested that all the potassium retained by isolated mitochondria is tightly bound to acceptor sites on the structural elements of the mitochondria. However, apart from the above considerations, this seems unlikely in view of Gamble's finding that only about one-tenth of the mitochondrial potassium remained associated with the fragments obtained after disrupting the mitochondria with digitonin.

These considerations suggest that the endogenous potassium of isolated mitochondria is largely in free solution in the non-accessible phase of the mitochondrial water, and that its concentration is probably over 100 m-molal. Therefore a true concentration difference of at least 60 m-molal potassium may have been maintained between mitochondria and medium during the first 20 min. of incubation at 21° (Table 1).

Permeability, adsorbency and volume of mitochondria. During the first 20 min. of incubation at 21° (Table 1) there were no changes in the water content of the mitochondrial pellets obtained on centrifuging. Therefore the volume of the mitochondria was constant during this period. The sucrose and chloride contents of the pellets were also constant, showing that the permeability of the mitochondria towards these two solutes did not alter. Nor did the potassium content of the pellets

vary, showing that there was no overall change in the adsorption of potassium by the mitochondria, or in the permeability of the mitochondria to potassium, i.e. there was no net flux of K^+ ions. Furthermore, the mitochondria maintained a concentration of potassium at least 35 m-molal higher than that in the medium. During this 20 min., 22% of the endogenous potassium of the mitochondria exchanged with labelled potassium from the medium. Hence, during this period, while the permeability, adsorbency and volume of the mitochondria were substantially constant, an exchange of K^+ ions took place, across a concentration difference of potassium. Ignoring for the moment the question of any electrical-potential difference, and considering only the chemical-concentration difference (see introductory paragraphs and papers there cited), it is possible to argue that during this period the exchange of K^+ ions was maintained by an active process.

Stanbury & Mudge (1953), Mudge (1955) and Gamble (1957) have also measured the exchange of ^{42}K between medium and mitochondria. However, they did not establish that a concentration difference of potassium was maintained between mitochondria and medium during the incubation. Nor did they obtain enough data to show that the results were not due to changes of mitochondrial volume, permeability or adsorbency. Instead they demonstrated that once the mitochondria had become labelled with ^{42}K , the radioactivity could not be removed by washing at 0° with solutions of sucrose, sodium chloride or potassium chloride. This provides valuable evidence (not obtained in the present experiment) that the ^{42}K had passed through a permeability barrier.

Effect of temperature. Although the results in Table 1 (at 21°) are not strictly comparable with those obtained at 0° (Amoore & Bartley, 1958), because of the different media, the rate of exchange of the endogenous potassium was about ten times as fast at the higher temperature (compare Mudge, 1955). A temperature coefficient (Q_{10}) of over 3 suggests either the presence of high potential-energy barriers (Danielli & Davson, 1935) or the participation of a metabolic process. Stanbury & Mudge (1953), Mudge (1955) and Gamble (1957) have demonstrated that the rate of exchange of mitochondrial potassium is closely related to the metabolic activity of the particles.

Swelling of mitochondria. In the experiment shown in Table 1, the mitochondria began to swell after 20 min. of incubation at 21° , became more permeable to sucrose and chloride and progressively lost their ability to maintain an excess concentration of potassium. Although an exchange of potassium continued, it was no longer possible to argue that active transport was taking place. The

exchange could have been due to the breakdown of the permeability barriers enclosing the previously inaccessible space, thus permitting the loss of endogenous potassium, and the entry of sucrose, chloride and labelled potassium from the medium.

When the mitochondria swelled (Table 1) it was the space already accessible to exogenous sucrose and chloride which swelled, and not the space which was initially inaccessible; the inaccessible space actually decreased in volume. Experiments described previously also show that when mitochondria swell, solutes from the medium enter the swollen volume (Bartley & Amoore, 1958; Amoore, 1958). These findings suggest that the swelling cannot be due to any internal osmotic pressure developed by crystalloids. The swelling of mitochondria, which occurs in certain unfavourable conditions, bears a marked resemblance to the so-called 'non-osmotic haemolysis' of erythrocytes described by Wilbrandt (1941). He considered that this form of haemolysis was caused by 'colloid osmotic pressure' developed by soluble protein within the erythrocyte. About half the protein of mitochondria is released in soluble form on disrupting the particles (Hogeboom & Schneider, 1950; Spector, 1953). Furthermore, the swelling of mitochondria can be prevented or reversed by adding colloids to the medium, whereas sucrose is ineffective (Lehninger, Ray & Schneider, 1959). Therefore isolated mitochondria may tend to swell if their limiting membranes become permeable to sucrose and salts while remaining impermeable to colloids.

Possible active transport. The experiment described above demonstrates that a preparation of isolated mitochondria is able to perform an exchange of K^+ ions across a concentration difference of potassium. Whereas it is basically similar to experiments described by other authors (see introductory paragraphs), it is felt that the modified technique meets the criticisms to which the earlier experiments might be subjected.

Nevertheless it has still not been proved that mitochondria are capable of active transport, because there is no knowledge of the potential difference across the mitochondrial membrane. The distribution of other ions (not estimated in this experiment) may have been such as to establish a potential difference of the appropriate sign and magnitude, so that the K^+ ions on each side of the membrane were actually in equilibrium, even though having different concentrations. The lower concentration of Cl^- ions inside the mitochondria than in the medium suggests that there may be a potential difference of the correct sign. The apparent concentrations of K^+ ions and Cl^- ions, in the phase of the mitochondrial water which was inaccessible to sucrose, were calculated for the

pellet obtained at time 25 min. from the start of the experiment. This calculation gave 180 m-molal K^+ ions and 17 m-molal Cl^- ions in the inaccessible space, compared with 42 m-molal potassium chloride in the medium. The concentration ratios were as follows:

$$\frac{K^+_{\text{inside}}}{K^+_{\text{outside}}} = 4.3. \quad \frac{Cl^-_{\text{outside}}}{Cl^-_{\text{inside}}} = 2.5.$$

These ratios are sufficiently close to each other, in view of the possibilities of error in estimation, to suggest that the distribution of K^+ and Cl^- ions might be determined by a Gibbs-Donnan equilibrium. Thus the K^+ and Cl^- ions may have been at equal electrochemical potential, and their distributions were not necessarily maintained by an active process.

The unequal distribution of K^+ and Cl^- ions may have been maintained by the presence of a high concentration of relatively indiffusible, negatively charged ions within the mitochondria. Werkheiser & Bartley (1957) showed that isolated mitochondria contain phosphate approximately equivalent, on a molar basis, to the potassium they contain, and that about 84 % of this phosphate is esterified. The mitochondrial membrane is considerably less permeable to adenosine 5'-phosphate and to adenosine triphosphate than to Na^+ ions (Amoore, 1958). The permeabilities to Na^+ and K^+ ions are similar (Amoore & Bartley, 1958). Hence it is probable that the endogenous organic phosphates of the mitochondria are indeed less capable of penetrating the mitochondrial membrane than are the K^+ ions. These findings favour the idea that the organic phosphates retained within the mitochondria may be able to support a large difference between internal and external concentrations of K^+ ions. In these circumstances an exchange of K^+ ions could take place, across a concentration difference of K^+ ions, and without any requirement for metabolic energy. Passage of the ions through the permeability barrier offered by the mitochondrial membrane demands only a sufficiency of potential energy (thermal energy), and does not require work to be done (Danielli & Davson, 1935).

Organic phosphate escapes from isolated mitochondria, during prolonged storage at 0°, or in response to treatment with orthophosphate (Amoore & Bartley, 1958; Amoore, 1958). This loss of organic phosphate is accompanied by a loss of K^+ ions as well. Presumably the mitochondrial membrane becomes permeable to the organic phosphate, which escapes, with the result that the mitochondria can no longer maintain a concentration of K^+ ions in excess of that in the medium. In the present experiment (Table 1), the net efflux of K^+ ions from the mitochondria, after the first 20 min.

of incubation at 21°, may have been due to the mitochondrial membrane becoming permeable to the endogenous organic phosphates.

SUMMARY

1. Isolated rat-liver mitochondria were incubated for 60 min. at 21° in 0.25 M-sucrose plus 0.04 M-potassium chloride containing ^{42}K . At intervals, samples of the suspension were centrifuged for analysis of the sedimented pellet and supernatant fluid. During the first 20 min. of incubation, the mitochondria maintained an internal concentration of potassium at least 35 m-molal higher than that in the medium. There were no changes in the permeability, adsorbency or volume of the mitochondria. In this time, 22 % of the endogenous potassium of the mitochondria exchanged with labelled potassium from the medium.

2. The exchange of potassium may have been due to active transport of K^+ ions into the mitochondria, but the participation of an active process was not definitely proved.

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