hydrogen-bonding in the ions concerned. They concluded that the combination of these effects enhances the acid strength of the 2-isomer more than that of the 1-isomer. The fact remains that the 2-isomer is the stronger acid above 18.9°, and consequently it must be assumed that other effects exist which make the 1-isomer the stronger acid below this temperature. The results obtained here serve as a warning of the dangers of using experimental pK values with a possible error of as much as ± 0.03 unit as a basis for conclusions about the magnitude and direction of effects such as the statistical effect and the inductive effect.

SUMMARY

1. The second dissociation constant of glycerol 1-phosphate has been determined over the range 5–50° at 5° intervals, cells without liquid junction with hydrogen and silver–silver chloride electrodes being used.

2. The standard thermodynamic quantities associated with the second dissociation of glycerol 1-phosphate, ΔG° , ΔS° , ΔH° and ΔC_p° have been evaluated and critically compared with those of glycerol 2-phosphate and orthophosphoric acid.

3. The information given will allow the preparation of standard buffers of glycerol 1-phosphate in the range pH 5–7, and up to ionic strengths of 0.1.

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The Permeability of Isolated Rat-Liver Mitochondria to Sucrose, Sodium Chloride and Potassium Chloride at 0°

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The distribution of solutes between isolated mitochondria and the incubation medium has been studied by several workers (Spector, 1953; Macfarlane & Spencer, 1953; Stanbury & Mudge, 1953; Bartley & Davies, 1954; Price, Fonnesu & Davies, 1956). The method is to separate the mitochondria by rapid centrifuging and analyse the sedimented pellet and supernatant fluid. From the distribution of solutes it has been inferred that certain ions are accumulated by the mitochondria,

and evidence has been presented connecting the accumulation with metabolism. However, the possible contribution by non-metabolic factors, such as permeability, adsorption, swelling or decomposition, was not always assessed before studying the effect of metabolism upon the distribution of solutes.

Werkheiser & Bartley (1957) have developed improved methods for studying the concentrations of solutes in mitochondria, and have emphasized

the value of conducting preliminary experiments at 0° in order to eliminate as far as possible the effects of metabolism upon the solute distribution. The low temperature also minimizes both the decomposition of labile substances and the swelling of mitochondria. Under these conditions the distribution of solutes should reflect the permeability and adsorbency of the mitochondria. Both Werkheiser & Bartley (1957) and Jackson & Pace (1956) have presented data on the permeability of rat-liver mitochondria, but the results are somewhat conflicting.

The aims of the work reported in this paper are to measure the water and solute content of rat-liver mitochondria isolated in 0.25 M-sucrose at 0°, and to study their permeability to sucrose, sodium chloride and potassium chloride at this temperature. The data obtained are intended to provide an adequate background for further studies of the effect of metabolism on solute movements.

METHODS

Preparation of mitochondria. Rat-liver mitochondria were prepared in 0.25 M-sucrose as described by Werkheiser & Bartley (1957). The final firmly packed mitochondrial residue was resuspended in 0.25 M-sucrose to make the 'stock suspension' which was kept at 0° until required (usually $\frac{3}{4}$ –1½ hr.).

Incubation. In all experiments described in this paper the mitochondrial suspensions were kept at 0° until the mitochondria had been separated from the medium by centrifuging for analysis. The incubation vessels were placed in an ice bath, and the contents occasionally gently shaken.

Sampling. The centrifuge tubes were previously chilled in ice, and the centrifuge was maintained at 0–2°. Samples of the mitochondrial suspension (2 ml., containing about 30 mg. of mitochondrial dry matter) were centrifuged at about 26 000 *g* for 10 min. The supernatant fluid was decanted into 1.0 ml. of 30% (w/v) trichloroacetic acid (TCA). The inside of the centrifuge tube was carefully dried with strips of filter-paper. After weighing, the pellet was extracted overnight with 1.0 ml. of 10% (w/v) TCA. In one experiment the mitochondria were centrifuged through silicone fluid of sp.gr. 1.055, according to the procedure of Werkheiser & Bartley (1957). (No lower layer of aqueous 'fixative' was used.)

Determination of mitochondrial dry matter. The TCA-extracted residues were washed and dried as described by Werkheiser & Bartley (1957), to obtain the weight of '10% TCA-insoluble mitochondrial dry matter' (*M*).

Estimation of extraparticulate fluid. The method of Werkheiser & Bartley (1957) was employed. [¹⁴C]Carboxypolyglucose (referred to below as 'labelled polyglucose') was added to the mitochondrial suspension in a final concentration of 0.5 or 1.0% (w/v). The sample of polyglucose was presented by Dr P. T. Mora, National Cancer Institute, National Institutes of Health, Bethesda, U.S.A., who has stated that it is a branched-chain polymer with spherical molecules, mol.wt. 50 000 (number-average).

Analytical methods

Sucrose. This was estimated by the method of Kulka (1956).

Phosphate. Total phosphate was estimated by the method of Berenblum & Chain (1938) as modified by Bartley (1953), after wet-ashing the sample by the method of Hanes & Isherwood (1949). Ashing was facilitated by the addition of 0.05 ml. of 5% (w/v) ammonium molybdate, as suggested by Werkheiser & Bartley (1957).

Chloride. The method of Sanderson (1952) was employed. A marked improvement in sensitivity and reproducibility was achieved by decreasing the volume of liquid in the titration vessel. A conical glass vessel, 3 cm. deep, of 1.0 ml. capacity was used. The titrant was 0.050 M-AgNO₃, delivered from an Agla micrometer syringe (Burroughs, Wellcome and Co.). The titration medium was 10% TCA, and a little capryl alcohol (octan-2-ol) was added to prevent frothing. The mitochondrial and supernatant extracts contained about 10% of TCA, and 0.05–0.2 ml. samples were titrated directly. With the smaller sample volumes 0.1 ml. of 10% TCA was added. The equivalence point was at 250 mv with 10% TCA as titration medium, compared with 220 mv when 0.1 N-HNO₃ was used. The silver electrode was cleaned as recommended by Werkheiser & Bartley (1957) before use.

Sodium and potassium. These elements were estimated by means of a lithium internal-standard flame photometer, which was built in this Laboratory. The design and operation of the instrument are described in the Appendix (Amoore, Parsons & Werkheiser, 1958).

Magnesium. The method of Mann & Yoe (1956) was adapted as follows: The sample (containing not more than 0.2 μ mole of magnesium) was diluted to 2 ml. with water, heated for 2 hr. in a boiling-water bath to decompose the TCA, cooled and again made up to 2.0 ml. with water. Then 0.1 ml. of 2 mM-CaCl₂, 1.0 ml. of magnesium reagent solution [0.015% (w/v) in redistilled ethanol] and 1.0 ml. of 0.08 M-sodium borate were added. [The magnesium reagent was sodium 1-azo-2-hydroxy-3-(2:4-dimethylcarboxanilido)naphthalene-1'-(2-hydroxybenzene-5-sulphonate), obtained from LaMotte Chemical Products Co., Baltimore, Md., U.S.A.] The volume was made up to 10 ml. with redistilled ethanol. The solution was mixed and allowed to stand for 1 hr. before the absorption was measured at 510 μ . Large amounts (75 μ moles) of sucrose decreased the colour intensity, but up to 25 μ moles/sample did not interfere. Accurate recoveries were obtained of Mg²⁺ added to mitochondrial extracts.

Radioactive potassium. ⁴²K₂CO₃ (A.E.R.E., Harwell, Berks) was neutralized to pH 7.4 with 0.125 N-HCl before addition to the mitochondrial suspension. An M6 liquid counter (Veall, 1948) was used. At least 10 000 counts were accumulated, and corrections were applied for background, dead-time and decay.

Calculations

External concentrations. The analyses of the supernatant were expressed in the same units as the internal concentrations (millimolal).

Internal concentrations. The method of calculating the internal water content of the mitochondria followed that used by Werkheiser & Bartley (1957). The internal concentrations of solutes were expressed in millimolal units. In

Table 1. *Extraparticulate fluid in mitochondrial pellets*

Labelled polyglucose was added as indicated to mitochondrial suspensions in 0.25 M-sucrose at 0°. Samples (2 ml.) were sedimented at 25 000 g for 10 min. The mitochondrial pellets were extracted with 10% trichloroacetic acid and assayed for labelled polyglucose. In this and other tables *M* represents mitochondrial dry wt.

Expt. no.	Concn. of labelled polyglucose (%)	Time of sedimentation after adding labelled polyglucose (min.)	Total water content of pellet (l./kg. of <i>M</i>)	Extra-particulate water (%)	Internal water content of mitochondria (l./kg. of <i>M</i>)
1	1.0	—	2.17	24.7	1.64
2	1.0	—	2.54	37.8	1.58
3	1.0	—	2.45	59.1	1.00
4	0.5	3	2.72	37.4	1.70
	0.5	90	2.73	33.6	1.80
5	1.0	5	2.10	53.5	0.98
	0.1	5	2.41	46.0	1.31
	0.01	5	2.41	35	1.5

some experiments the amount of extraparticulate fluid was not estimated. The total water content of the pellet was therefore calculated instead, and the concentration of solutes in the pellet expressed on this basis.

Solute 'spaces'. The solute 'space' of the mitochondria, considered to be accessible to an external solute, was the ratio of the internal and external concentrations of the solute, expressed as a percentage. When the extraparticulate fluid was not estimated, the solute space of the pellet was calculated instead.

Non-accessible volume. The volume of mitochondrial water considered to be non-accessible to an external solute was calculated and expressed as l./kg. of *M*.

Solute content. The number of moles of a solute, divided by *M*, gave the solute content. This was expressed as m-moles/kg. of *M*.

RESULTS

Estimation of extraparticulate fluid by means of labelled polyglucose. Several preparations of mitochondria in 0.25 M-sucrose were made, 1.0% of labelled polyglucose was added to the suspension, and samples were centrifuged at 0°. Experiments 1–3 (Table 1) show wide variation in the proportion of water which was extraparticulate, from 24 to 59% in different preparations. Using the same method, Werkheiser & Bartley (1957) found that 19–22% of the water was extraparticulate. Jackson & Pace (1956) have also measured the extraparticulate fluid in mitochondrial pellets by using ¹³¹I-labelled albumin. They found that about 18% of the volume of the pellet was extraparticulate fluid. The use of labelled polyglucose might yield an overestimate of the extraparticulate fluid, due to either slow penetration into the particles or adsorption on their surface.

The possibility that labelled polyglucose might slowly penetrate the mitochondria was tested in Expt. 4 (Table 1). Labelled polyglucose in a final concentration of 0.5% was added to a mitochondrial

suspension in 0.25 M-sucrose. A sample was withdrawn and sedimented 3 min. after mixing, and another sample after a further 87 min. storage at 0°. During this period the total water content of the pellet was almost unchanged, but the percentage of extraparticulate water decreased from 37 to 33%. There was an increase in the internal water content of the mitochondria from 1.70 to 1.80 l./kg. of *M*. This showed that at the end of the experiment labelled polyglucose was excluded from a greater volume than at the start, and so it did not penetrate the mitochondria during the interval.

The alternative that labelled polyglucose might be adsorbed by the mitochondria was tested in Expt. 5 (Table 1). Labelled polyglucose was added to three samples of mitochondrial suspension in final concentrations of 1.0, 0.1 and 0.01%, and the samples were centrifuged simultaneously. It was expected that, if adsorption occurred, the use of a low concentration of labelled polyglucose would yield an overestimate of the extraparticulate fluid. However, this did not happen, which suggests that no adsorption had occurred.

Jackson & Pace (1956) applied a different test of the possibility that their labelled colloid might be adsorbed by the mitochondria. They added the [¹³¹I]albumin to a suspension of mitochondria in 0.25 M-sucrose, centrifuged and resuspended the residue in fresh sucrose. After centrifuging again, they found that only about three-quarters of the radioactivity had been removed from the original mitochondrial pellet. This experiment has been repeated, with labelled polyglucose, and similar results were obtained. However, Expt. 5, Table 1, showed that the retention of labelled polyglucose in the pellet could not be due to adsorption. It was noted that on resuspending the residue some small flakes of the pellet remained visible. The failure to

achieve the expected dilution of radioactivity on resuspension was probably due to incomplete dispersion of the mitochondria and consequent entraining of labelled polyglucose.

Sucrose content of isolated mitochondria. Several preparations of rat-liver mitochondria were made in 0.25M-sucrose, and samples were sedimented at 0° for analysis. The results in Expts. 1-3 (Table 2) showed a wide variation from one preparation to another in the internal concentration of sucrose, which was 80-200 millimolal, but was always less than the external concentration. A partial permeability of mitochondria to sucrose has been observed by Jackson & Pace (1956) and also by Werkheiser & Bartley (1957), who reported values of 57-59% for the sucrose 'space' of mitochondria isolated by the same procedure. In the present work the sucrose space varied from 34 to 74% (Table 2).

Potassium content of isolated mitochondria. The internal concentration of potassium (Table 2) varied from 24 to 94 millimolal. Werkheiser & Bartley (1957) found 47-59 millimolal potassium in isolated mitochondria. In the present series of experiments the potassium content was 38-138 m-moles/kg. of *M*. Price *et al.* (1956) found 113 m-moles of potassium/kg. of dried mitochondrial pellet, sedimented from 0.25M-sucrose at 0°.

When the internal concentrations of sucrose and potassium in Expts. 1-3 (Table 2) were compared, it was noted that a high sucrose concentration was associated with a low potassium concentration and vice versa. It was considered that this inverse relationship might be due to a gradual loss of potassium from the mitochondria during storage of the stock suspension, and replacement by sucrose. About $\frac{3}{4}$ -1 $\frac{1}{4}$ hr. elapsed in most experiments between the completion of the mitochondrial preparation and sampling the stock suspension.

To test the stability of a stock suspension, it was sampled 1 $\frac{1}{4}$ hr. after the preparation had been completed, and again after a further 87 min. storage at 0°. The analyses are shown in Expt. 4 (Table 2). During this period the internal potassium concentration decreased by 20%, and the sucrose concentration increased by 25%. A slight increase in water content also occurred, but it amounted only to 6%.

Changes of solute content of mitochondria during prolonged storage in 0.25M-sucrose at 0°. In view of

Fig. 1. Changes of solute content of mitochondria during prolonged storage in 0.25M-sucrose at 0°. *M* represents mitochondrial dry wt. (see Methods). The incubation and sampling procedure are described in the text. ●, Potassium content; ▲, trichloroacetic acid-extractable phosphate content; ■, magnesium content; ○, water content; △, volume of mitochondrial water non-accessible to sucrose.

Table 2. Concentrations of sucrose and potassium in isolated mitochondria

Samples (2 ml.) of mitochondrial suspensions in 0.25M-sucrose at 0° (containing labelled polyglucose) were sedimented at 25 000 *g* for 10 min. The mitochondrial pellets and the supernatant fluid were analysed for labelled polyglucose, sucrose and potassium.

Expt. no.	Internal water content of mitochondria (l./kg. of <i>M</i>)	Sucrose (millimolal)		Sucrose 'space' of mitochondria (%)	Vol. of mitochondrial water non-accessible to sucrose (l./kg. of <i>M</i>)	Potassium (millimolal)		Potassium content (m-moles/kg. of <i>M</i>)
		External	Internal			External	Internal	
1	0.99	233	80.1	34.4	0.65	0.2	94.1	93.3
2	1.64	250	132	52.7	0.77	0.8	52.8	86.5
3	1.58	264	200	74.7	0.38	1.1	24.0	38.0
4	1.70	247	136	55.3	0.76	0.4	61.0	103
*	1.80	247	170	69.0	0.56	0.6	48.9	88.5

* Sedimented 87 min. later.

the considerable changes in sucrose and potassium content which occurred during storage for $1\frac{1}{2}$ hr. of the stock suspension (Table 2, Expt. 4), the changes occurring over a much longer period were studied. A stock suspension of mitochondria was prepared as rapidly as possible ($2\frac{1}{4}$ hr. from the death of the rats) by omitting the resuspension and recentrifuging of the nuclear fraction. The stock suspension in 0.25M-sucrose was kept at 0° and sampled at 2 hr. intervals for 10 hr. Each sample (2 ml.) was mixed with 0.1 vol. of 10% (w/v) labelled polyglucose and allowed to stand at 0° for 5 min. before it was centrifuged. The results are shown in Fig. 1. The content of potassium decreased at a uniform rate from 130 to 76 m-moles/kg. of M during the first 4 hr., but then remained constant for the next 4 hr., after which a further small loss occurred. The curve for total TCA-extractable phosphate content was similar, decreasing from 117 to 92 m-moles/kg. of M during the first 4 hr., with little further change except for a slight fall at the end. The magnesium content was initially 37 m-moles/kg. of M and decreased very slightly during the first 4 hr. The chloride content remained at about 16 m-moles/kg. of M , and the sodium content was less than 6 m-moles/kg. of M (not shown).

The volume of mitochondrial water which was non-accessible to sucrose was initially 0.81 l./kg. of M and decreased at a uniform rate for the first 4 hr. to 0.55 l./kg. of M . There was no change for the next 2 hr., but during the last 4 hr. a further decrease to 0.48 l./kg. of M took place. During the first 4 hr. the rate of loss (in m-moles/kg. of M /hr.) of potassium was 12.6, of phosphate 5.5 and of magnesium 0.7. During the same period the rate of penetration of sucrose into the initially non-accessible volume was 14.7 m-moles/kg. of M /hr.

This experiment confirmed the reciprocal relationship previously noted between the movements of potassium and sucrose. However, the actual rate of the process could not account for the range of values of potassium content given in Table 2, as the variation in the time taken to prepare the mitochondria was not greater than 1 hr., during which a loss of about 12 m-moles/kg. of M might be expected, whereas the potassium content of different mitochondrial preparations varied over a range of 100 m-moles/kg. of M .

Solute content of the residues obtained at each stage of the preparation of mitochondria. Until the final stage of the preparations, the mitochondria were accompanied by a certain amount of 'fluffy layer' which sedimented above them. This was freely flowing and could be partially removed by pouring it off with the supernatant fluid. The fluffy layer is usually considered to be a mixture of microsomal and mitochondrial material (Novikoff, Podber,

Ryan & Noe, 1953). Werkheiser & Bartley (1957) showed that mitochondria could be centrifuged through silicone fluid of sp.gr. 1.055. It seemed unlikely that the fluffy material would be dense enough to penetrate this fluid, and therefore this procedure might afford a means of fractionating the particles. Accordingly, a mitochondrial preparation (omitting the resuspension and recentrifuging of the nuclear fraction) was carried through, and samples of the suspension were removed at each stage for sedimentation in a second refrigerated centrifuge. The suspension (4.5 ml.) was mixed with 0.5 ml. of 10% (w/v) labelled polyglucose and two 2 ml. samples were sedimented simultaneously, one directly and the other through 2.5 ml. of silicone fluid of sp.gr. 1.055. The centrifuge was accelerated maximally and run for 10 min. at 26 000 g.

The mitochondrial suspension was sampled at four stages in the preparation. At each stage the residue which penetrated the silicone fluid resembled the mitochondrial pellets normally obtained at the end of a preparation, having a pale buff colour without any pink fluffy layer. In all stages except the last, some pink material remained at the interface between the supernatant medium and the silicone.

During the fractionation the water content of the 'total particles' (sedimented without silicone) decreased from 7.5 l./kg. of M in the first residue to 1.00 l./kg. of M in the final preparation (Table 3, stages 1 and 4). The final preparation contained much higher concentrations of potassium, phosphate and magnesium, and a much lower concentration of sucrose, than the first residue. The particles obtained at the second and third stages were intermediate in composition. These results implied that the first three residues consisted of mixtures of particles. This conclusion was supported by the analyses of the particles which penetrated the silicone fluid (the 'dense particles'). The water content of the particles which penetrated the silicone was lower than that of the total particles at each stage except the last, where it was the same. At this stage also, the whole of the particulate dry matter penetrated the silicone, which did not occur with the earlier samples. In the first three stages of the preparation, the dense particles contained higher potassium, phosphate and magnesium concentrations than the total particles, but a lower sucrose concentration. In the final preparation there was little difference between the composition of the total particles and the dense particles.

These results showed that the mitochondria obtained in the final preparation, characterized by their high potassium, phosphate and magnesium concentration and low water and sucrose content, were accompanied in the earlier stages of the preparation by diminishing proportions of particles

Table 3. *Distribution of solutes during the preparation of mitochondria*

Duplicate samples (2 ml.) of the suspension of particles in 0.25 M-sucrose (containing 1% of labelled polyglucose) at 0° were centrifuged, one directly and the other through silicone fluid (sp.gr. 1.055) at 25 000 g for 10 min. The 'dense' particles are those which penetrated the silicone fluid.

Stage of preparation	Sample analysed	TCA-insoluble dry matter (mg.)	Internal water content (l./kg. of M)	Concns. of solute (millimolal)				
				Sucrose	Potassium	TCA-extractable phosphate	Magnesium	Sodium
1. Crude mitochondrial residue	Supernatant	—	—	189	19.4	9.0	1.25	6.4
	Total particles	32.4	7.5	157	33.6	9.8	5.9	8.6
	Dense particles	14.6	4.1	127	38.7	19.3	10.3	9.9
2. Once-washed mitochondria	Supernatant	—	—	225	3.8	1.6	0.2	1.3
	Total particles	26.1	4.9	174	26.2	15.7	9.5	4.0
	Dense particles	8.7	2.3	149	59.6	43.1	19.4	4.8
3. Twice-washed mitochondria	Supernatant	—	—	233	0.6	0.3	<0.2	0.2
	Total particles	12.8	2.44	146	47.4	40.7	18.1	1.3
	Dense particles	10.9	1.64	124	77.0	58.1	23.9	7.6
4. Final mitochondrial preparation	Supernatant	—	—	236	0.2	0.2	<0.2	<0.1
	Total particles	13.7	1.00	31.5	120	109	37.3	3.3
	Dense particles	13.9	1.00	14.4	116	95.8	33.1	3.3
								15.8

poor in endogenous solutes and with high water and sucrose contents.

Appearance of the mitochondrial preparations in the electron microscope. Through the courtesy of Dr G. A. Meek of the Department of Human Anatomy, Oxford University, a number of our preparations of mitochondria have been examined with the Siemens Elmiskop 1. The methods of fixing and sectioning the mitochondria were based on the procedures described by Witter, Watson & Cottone (1955). The general appearance of the micrographs was similar to those published by these authors of rat-liver mitochondria isolated in 0.25 M-sucrose. There was no sign of contamination by 'microsomal' or other particulate material. Some of the mitochondria contained 'vesicles', as described by Witter *et al.* (1955), although the extent of this damage was not so great as that noted by them. Preparations containing a high proportion of vesiculated mitochondria had a higher water content and sucrose space, and lower potassium and phosphate concentrations, than those showing less vesiculation.

The heterogeneity of the preparations of mitochondria, with respect to endogenous solute content and permeability to sucrose, would account for the wide range of analyses which have been found for different preparations of mitochondria. It should also be borne in mind when the distribution of sucrose, sodium chloride and potassium chloride is considered in the following sections.

Effect of 0.125 M-sodium chloride on the solute content of mitochondria. A concentration of 0.125 M-sodium chloride was chosen because it has the same osmolality as 0.25 M-sucrose. A 4 ml. sample of a suspension of mitochondria in 0.25 M-sucrose was centrifuged and the supernatant poured off. The residue was resuspended in 4 ml. of ice-cold 0.125 M-NaCl and kept at 0° for 15 min. A 2 ml. sample was then centrifuged at the same time as a 2 ml. sample of the original suspension in 0.25 M-sucrose. The analyses of the mitochondrial pellets and supernatant fluid are shown in Table 4. In Expt. 1 the pellet sedimented from 263 millimolal sucrose contained 182 millimolal sucrose, indicating a value of 69% for the sucrose space of the pellet, or 0.79 l./kg. of M for the volume of mitochondrial water which was non-accessible to sucrose. The supernatant from the second sample (sedimented from 0.125 M-sodium chloride) contained 12 millimolal sucrose, while the pellet contained only 11 millimolal sucrose. Therefore more than nine-tenths of the sucrose content of the original pellet was removed by resuspending in saline and recentrifuging. However, the sucrose content of the pellet did not decrease quite so much as would be expected if the sucrose space remained the same. This may be due to entrainment

of sucrose in fragments of the pellet, as happened with the labelled polyglucose.

As compensation for the removal of sucrose from the pellet, there were large increases in the sodium and chloride contents, but neither ion reached as high a concentration in the pellet as was present in the supernatant. In the first experiment the sucrose, sodium and chloride spaces of the pellets were respectively 69, 73 and 65 %, and in the second experiment 63, 63 and 60 %. This indicated that the mitochondria were about equally permeable to these solutes. Conversely, about 0.8–0.9 l./kg. of *M* of mitochondrial water remained non-accessible to sucrose, sodium or chloride.

It was noted that, when the mitochondria sedimented from sodium chloride were compared with those sedimented from sucrose, the increase in sodium concentration in each was greater than the increase in chloride concentration. In Expt. 1 (Table 4) the sodium concentration in the pellet increased by 87 millimolal, while the chloride concentration only increased by 74 millimolal. The corresponding figures in Expt. 2 were 79 millimolal for sodium and 70 millimolal for chloride. In the first experiment there was a small loss of potassium from the pellet, but in the second experiment it remained almost unchanged. The concentration of total TCA-extractable phosphate was unchanged

by the saline medium, being 38 millimolal in Expt. 1 and 53 millimolal in Expt. 2, but the magnesium concentration decreased from 11.4 to 8.6 millimolal in Expt. 1 and from 12.4 to 10.4 millimolal in Expt. 2.

Effect of 0.125 M-potassium chloride on the solute content of mitochondria. The experiments described in the preceding section were repeated, with the same stock suspensions of mitochondria, but with 0.125 M-potassium chloride instead of sodium chloride. The results are shown in Table 5. In both experiments most of the sucrose was replaced by potassium chloride. The distribution of chloride was very similar, whether it was added as the potassium or sodium salt (compare Tables 4 and 5).

When the pellets sedimented from potassium chloride were compared with those sedimented from sucrose, it was found that the increase in potassium concentration was somewhat greater than the increase in chloride concentration. In Expt. 1 (Table 5) the potassium concentration in the pellet increased by 85 millimolal, while the chloride increased by only 64 millimolal. In Expt. 2 the figures were 88 and 72 millimolal respectively.

In Expt. 2 (Table 5) a tracer amount of radioactive potassium chloride (^{42}KCl) was included in the resuspension medium. From the specific activity of the potassium in the supernatant and in

Table 4. *Effect of 0.125 M-sodium chloride on the solute content of mitochondria*

A suspension of mitochondria in 0.25 M-sucrose (4 ml.) was sedimented, and the residue resuspended in 4 ml. of 0.125 M-NaCl at 0°. Samples (2 ml.) of this suspension and of the original suspension in 0.25 M-sucrose were centrifuged for analysis of pellet and supernatant. Sample (a) was the sucrose suspension, and sample (b) the NaCl suspension, in each experiment.

Expt. no.	Total water content of pellet (l./kg. of <i>M</i>)	Concns. of solute (millimolal)							
		Sucrose		Sodium		Chloride		Potassium	
		S.*	P.*	S.	P.	S.	P.	S.	P.
1a	2.54	263	182	<0.2	0.4	<0.1	4.3	0.3	42.5
b	2.55	12.0	11.2	121	88.1	120	78.1	0.4	38.4
2a	2.55	268	170	<0.1	<0.4	<0.1	5.3	0.2	54.1
b	2.48	9.2	7.5	124	79.0	126	75.0	0.5	55.0

* S., supernatant; P., pellet.

Table 5. *Effect of 0.125 M-potassium chloride on the solute content of mitochondria*

Details as in Table 4, except that 0.125 M-KCl was used instead of NaCl. The mitochondrial suspensions in 0.25 M-sucrose were the same as those used in Table 4. In Expt. 2b, a trace amount of ^{42}KCl was included in the medium.

Expt. no.	Total water content of pellet (l./kg. of <i>M</i>)	Concns. of solute (millimolal)						
		Sucrose		Potassium		^{42}K in pellet	Chloride	
		S.*	P.*	S.	P.		S.	P.
1a	2.54	263	182	0.3	42.5	—	<0.1	4.3
b	2.54	8.9	8.2	122	128	—	118	68.7
2a	2.55	268	170	0.2	54.1	—	<0.1	5.3
b	2.42	8.6	7.9	123	142	88.0	125	77.7

* S., supernatant; P., pellet.

the mitochondrial pellet it was concluded that the labelled potassium reached a concentration of only 88 millimolal in the pellet, whereas the total potassium concentration was 142 millimolal. The difference (unlabelled potassium) was 54 millimolal, which was the same as the concentration of endogenous potassium in the pellet sedimented from 0.25 M-sucrose. The endogenous potassium was therefore retained by the mitochondria on resuspension in 0.125 M-potassium chloride, and failed to exchange with the potassium of the medium. The concentration of labelled potassium in the mitochondrial pellet was used to calculate the potassium space of the pellet, which was 71 %. This was a little larger than the corresponding sucrose and chloride spaces of 63 and 62 %, or the sodium space of 63 % found in the experiment with sodium chloride. It indicated that about 0.7 l./kg. of *M* of the mitochondrial water was non-accessible to external K^+ ions.

The concentration of TCA-extractable phosphate was not altered by resuspending the mitochondrial pellet in 0.125 M-potassium chloride and resedimenting, but the magnesium concentration was lowered to the same levels as were observed with the sodium chloride resuspension medium.

The removal of sucrose from a preparation of mitochondria was further studied. A stock suspension of mitochondria in 0.25 M-sucrose was maintained at 0°. At the start, and after 4½ and 8½ hr., two samples (2 ml.) of the suspension were centrifuged. One of each pair of residues was resuspended in 2 ml. of ice-cold 0.125 M-potassium chloride. After they had stood for 5 min. at 0°, the mitochondria were again sedimented. The analyses are shown in Table 6, Expts. 1-3. Although the sucrose concentration in the control residues sedi-

mented from 0.25 M-sucrose increased during storage of the stock suspension, almost all of it was washed out on resuspension in potassium chloride solution, leaving only a small excess concentration of sucrose in the pellet. The distribution of sucrose and chloride was used to calculate the volume of water in the pellets which could be regarded as being non-accessible to these solutes. The results are shown in the last two columns of Table 6. For each pair of pellets the volume non-accessible to chloride in the resuspended pellet was similar to that non-accessible to sucrose in the control pellet.

In these experiments, which showed almost complete removal of sucrose from the mitochondria, 5-15 min. elapsed between resuspending the mitochondria in the saline medium and sedimenting them again. A shorter delay was achieved by mixing a concentrated suspension of mitochondria in 0.25 M-sucrose with four times its volume of 0.125 M-potassium chloride, and centrifuging a sample immediately. The results are shown in Expt. 4 (Table 6). Less than 2 min. elapsed between mixing the fluids and starting the centrifuge, yet the mitochondrial sucrose had already equilibrated with the new suspension medium, and analysis of a further pellet sedimented 18 min. later showed little further change in solute content. These experiments show that, although the penetration of sucrose into the mitochondria may take many hours, its removal may be achieved in a few minutes.

Changes of solute content of mitochondria during prolonged storage in 0.125 M-sodium chloride and in 0.125 M-potassium chloride at 0°. Three samples of the same suspension of mitochondria in 0.25 M-sucrose were centrifuged, and the pellets were

Table 6. Removal of sucrose from mitochondrial pellets by washing with 0.125 M-potassium chloride

In Expts. 1-3 a suspension of mitochondria in 0.25 M-sucrose was kept at 0°. At the stated times samples (2 ml.) were centrifuged. One residue was resuspended in 2 ml. of ice-cold 0.125 M-KCl and resedimented. In each experiment sample (a) was sedimented from 0.25 M-sucrose, and sample (b) from 0.125 M-KCl. In Expt. 4, 1 ml. of a concentrated suspension of mitochondria in 0.25 M-sucrose was added to 4 ml. 0.125 M-KCl. Samples (2 ml.) were centrifuged.

Expt. no.	Time	Total water content of pellet (l./kg. of <i>M</i>)	Concns. of solute (millimolal)						Vol. of water in pellet (l./kg. of <i>M</i>) non-accessible to	
			Sucrose		Potassium		Chloride			
			S.*	P.*	S.	P.	S.	P.	Sucrose	Chloride
1a	0	3.17	244	178	0.1	41.0	0.1	5.4	0.81	—
b		3.16	7.1	9.4	128	141	126	97.9	—	0.70
2a	4½ hr.	2.50	245	186	0.3	30.4	0.1	7.3	0.55	—
b		3.14	4.5	13.6	132	139	128	106	—	0.54
3a	8½ hr.	2.56	245	192	0.5	29.0	0.1	5.0	0.52	—
b		3.15	3.9	12.2	131	136	130	106	—	0.58
4	2 min.	2.50	49.4	37.0	107	108	—	—	0.62	—
	18 min.	2.46	49.5	35.7	106	110	—	—	0.68	—

* S., supernatant; P., pellet.

resuspended in 0.25M-sucrose, 0.125M-sodium chloride and 0.125M-potassium chloride respectively. The suspensions were kept at 0° with occasional gentle shaking, and samples were withdrawn for centrifuging and analysis. The analyses of the pellets sedimented from each suspension at the start of the experiment have already been given in Table 4 (Expt. 2) and Table 5 (Expt. 2).

The results of storage for 8 hr. at 0° of the suspension in 0.125M-sodium chloride are shown in Table 7. During the 8 hr. there was a slow increase in the sodium and chloride concentrations in the pellet (16 and 14 millimolal respectively), but neither of these ions reached as high a concentration in the pellet as that in the medium. In the same interval there was a loss from the pellet of 18 millimolal potassium, which was slightly more than equivalent to the sodium which penetrated. The total TCA-extractable phosphate concentration also decreased, but only by 6 millimolal.

Table 8 shows the results of the experiment in which the suspension medium was 0.125M-potassium chloride. A trace amount of ^{42}KCl was included in the medium. The only appreciable change that occurred during the experiment was a

steady increase in specific activity of the potassium in the pellet. Whereas initially none of the endogenous potassium exchanged, at the end of the experiment about 75 % of it had reached the same specific activity as the potassium in the medium.

The suspension in 0.25M-sucrose was sampled only at the start (see Table 4, Expt. 2a) and after storage for 8 hr. at 0°. In this interval the water content of the pellet decreased slightly from 2.55 to 2.31 l./kg. of *M*, but the magnesium content was unaltered at 32 m-moles/kg. of *M*, and the sucrose concentration increased from 170 to 198 millimolal. The potassium concentration decreased from 54 to 23 millimolal, while the TCA-extractable phosphate concentration fell from 52 to 39 millimolal. The losses of potassium and of phosphate from the pellet were about twice as rapid in 0.25M-sucrose as they were in 0.125M-sodium chloride.

Effect of mixtures of 0.25M-sucrose and 0.125M-potassium chloride on the solute content of mitochondria. A concentrated suspension of mitochondria in 0.25M-sucrose (1 ml.) was added to 4 ml. of medium, which consisted of 0.25M-sucrose or 0.125M-potassium chloride, or mixtures of these

Table 7. *Effect of storage in 0.125M-sodium chloride at 0° on the solute content of mitochondria*

A suspension of mitochondria in 0.25M-sucrose (16 ml.) was sedimented, and the residue resuspended in 16 ml. of ice-cold 0.125M-NaCl. The suspension was kept at 0°, with occasional gentle shaking. Samples (2 ml.) were centrifuged for analysis as indicated. The concn. (millimolal) of sodium in the supernatant was found to be 127, chloride 126 and sucrose 9.1. During the experiment, the concn. of potassium in the supernatant increased from 0.5 to 1.6 millimolal.

Time (hr.)	Total water content of pellet (l./kg. of <i>M</i>)	Concns. of solute in pellet (millimolal)					TCA- extractable phosphate	Magnesium
		Sucrose	Sodium	Potassium	Chloride			
0	2.48	7.5	79.0	55.0	75.0	54.3	10.4	
$\frac{1}{2}$	2.42	7.2	80.1	55.4	77.0	53.6	10.8	
1	2.49	7.1	88.1	51.0	78.9	53.9	10.3	
2	2.45	7.1	90.4	44.3	83.0	50.0	10.9	
4	2.36	7.2	96.0	42.1	84.8	49.7	10.8	
8	2.38	7.0	95.0	36.5	89.0	48.4	11.6	

Table 8. *Effect of storage in 0.125M-potassium chloride at 0° on the solute content of mitochondria*

Details as in Table 7, except that 0.125M-KCl was used instead of NaCl. The mitochondrial preparation was the same as that used in Table 7. A trace amount of ^{42}KCl was included in the medium. The concn. (millimolal) of potassium in the supernatant was 123, chloride 125 and sucrose 8.6. There was <2 millimolal sodium in the pellets and <1 millimolal sodium in the supernatant.

Time (hr.)	Total water content of pellet (l./kg. of <i>M</i>)	Concns. of solute in pellet (millimolal)						TCA- extractable phosphate	Magnesium
		Sucrose	Total potassium	^{42}K	Unex- changed potassium	Chloride			
0	2.42	7.9	142	88.0	54	77.7	52.3	10.1	
$\frac{1}{2}$	2.33	7.3	148	88.0	60	78.0	55.1	10.8	
1	2.36	6.5	148	96.7	51	76.8	54.2	10.8	
2	2.40	6.7	145	106	39	79.0	53.4	10.6	
4	2.36	6.1	145	118	27	79.9	52.2	10.6	
8	2.50	6.0	146	132	14	84.1	49.8	9.9	

two solutions. Labelled polyglucose was included in the mixture at a final concentration of 1%. After being kept at 0° for 15 min., two 2 ml. samples were withdrawn for sedimentation. The analysis of the mitochondria centrifuged from 0.25M-sucrose (Expt. 1, Table 9) shows that this preparation of mitochondria had an unusually high sucrose concentration and low potassium concentration. When potassium chloride was included in the medium, the water content of the mitochondria became about 25% higher than that in the control samples without potassium chloride. It is not understood why the addition of potassium chloride to the sucrose medium resulted in an increase in mitochondrial volume. There was no difference between the effect of 0.002M- and 0.1M-potassium chloride in this respect.

In all samples the sucrose concentration in the mitochondria was lower than that in the supernatant. The mitochondria sedimented from 0.25M-sucrose contained 7 millimolal chloride. The internal chloride concentration did not increase until 11 millimolal chloride was added to the medium. With higher concentrations of chloride in the medium the concentration of chloride in the mitochondria was less than that in the medium.

The potassium concentration in the mitochondria sedimented from 0.25M-sucrose was 24 millimolal (Expt. 1, Table 9). In Expts. 2-6 concentrations of potassium up to 46 millimolal were present in the medium. These resulted in increases in the internal concentration which were roughly equal to the increases in the potassium concentration of the medium. Thus the mitochondria maintained an excess concentration of 19-22 millimolal potassium above the medium, when the latter contained 1-46 millimolal potassium. These findings are consistent with the view expressed by Werkheiser & Bartley (1957) that under these conditions the endogenous potassium of the mitochondria remained bound, while the added potassium became

distributed throughout all the mitochondrial water. However, when an external potassium concentration of 107 millimolal was tested (Expt. 7), the excess concentration in the mitochondria fell to 14 millimolal.

The experiment showed that there was consistently an exclusion of sucrose and of chloride from part of the mitochondrial volume. The excess uptake of potassium compared with chloride, which has been previously noted, was observed with all external concentrations of potassium chloride.

Effect of hypertonic sucrose and mixtures of sucrose and potassium chloride on the water and solute content of mitochondria. A concentrated suspension of mitochondria in 0.25M-sucrose (1 ml.) was added to 4 ml. of ice-cold 0.25M-sucrose; then the appropriate volume of 2.5M-sucrose or 1.5M-potassium chloride was added slowly, with mixing. The stock suspension of mitochondria was the same as that used in the experiment described in the preceding section, and the concentration of labelled polyglucose and the sampling procedure were the same. In Table 10, Expt. 1, the mitochondria were sedimented from 0.25M-sucrose without addition. The addition of equal osmolar amounts of sucrose or potassium chloride produced the same decrease in the water content of the mitochondria (compare Expts. 2 with 5, 3 with 6, and 4 with 7). Throughout this series (Expts. 1-7) there was an almost constant ratio between the internal and external concentrations of sucrose, indicating that a constant volume (about 0.4 l./kg. of *M*) remained inaccessible to sucrose.

The samples of Expts. 2-4 were centrifuged together 30 min. later than the control (Expt. 1). The observed concentrations of potassium were the result of different amounts of leakage of potassium, counter-balanced by decreases of water content due to the hypertonic medium which increased the concentration of internal solutes. In Expts. 5-7 there was an exclusion of chloride, whereas the

Table 9. *Effect of mixtures of 0.25M-sucrose and 0.125M-potassium chloride on the solute content of mitochondria*

A concentrated suspension of mitochondria in 0.25M-sucrose (1 ml.) was added to 4 ml. of 0.25M-sucrose or 0.125M-KCl or mixtures of the two. Labelled polyglucose (1%) was included in the medium. After 15 min. at 0°, two samples (2 ml.) were sedimented for analysis.

Expt. no.	Internal water content of mitochondria (l./kg. of <i>M</i>)	Concns. of solute (millimolal)					
		Sucrose		Potassium		Chloride	
		External	Internal	External	Internal	External	Internal
1	1.58	264	200	1.2	24.0	0.1	7.0
2	1.96	261	223	3.6	24.2	2.5	5.9
3	2.02	251	222	6.4	26.1	5.5	6.4
4	2.10	248	212	11.8	31.0	11.2	11.0
5	2.00	219	190	22.4	44.8	22.0	20.6
6	1.98	173	155	46.1	65.0	44.3	38.0
7	2.21	50.2	41.5	107	121	107	86.0

potassium analyses were in agreement with the postulate that this ion had penetrated all the internal water of the mitochondria, without loss of that potassium which was initially present.

The results of two more experiments are included in Table 10, in which the analyses are given for different preparations of mitochondria in 0.25M-sucrose (Expts. 8a and 9a) and for mitochondria obtained after adding potassium chloride in a final concentration of approximately 0.04M to the medium (Expts. 8b and 9b), which may be compared with Expt. 6. In Expts. 8b and 9b the addition of 0.04M-potassium chloride produced shrinkages of the mitochondria of 15 and 21% respectively, which was rather more than that in Expt. 6 (9%). The salt altered the distribution of the sucrose in such a way that the volume which remained inaccessible to sucrose showed a considerable decrease, from 0.77 to 0.48 l./kg. of *M* in Expt. 8 and from 0.76 to 0.66 l./kg. of *M* in Expt. 9.

Effect of a mixture of sucrose and sodium and potassium chlorides on the solute content of mitochondria. The conditions of this experiment were

based on those used by Werkheiser & Bartley (1957) when they measured the distribution of sodium, potassium and chloride ions between mitochondria and the medium. They added sodium chloride and potassium chloride together to a suspension of mitochondria in 0.25M-sucrose, so that the final concentration of each salt was 0.04M. In the present experiment (see Table 11), four samples (2 ml.) of a suspension of mitochondria in 0.25M-sucrose received respectively no addition, 0.04M-sodium chloride, 0.04M-potassium chloride, and 0.04M-sodium chloride plus 0.04M-potassium chloride. After standing for 10 min. at 0° they were centrifuged for analysis.

The addition of salts decreased the water content of the mitochondrial pellets. In Expts. 2-4 (Table 11) the concentration of chloride in the pellet was always considerably less than that in the supernatant. In Expts. 2 and 4 the concentration of sodium in the pellet also remained below that in the supernatant, but the sodium space in the pellet was in each case larger than the chloride space. In Expts. 3 and 4 the concentration of potassium

Table 10. *Effect of hypertonic media on the water and solute content of mitochondria*

In Expts. 1-7 a concentrated suspension of mitochondria in 0.25M-sucrose (1 ml.) was added to 4 ml. of ice-cold 0.25M-sucrose. 2.5M-sucrose or 1.5M-KCl was added. The mitochondrial suspension and sampling were the same as in Table 9. Labelled polyglucose (1%) was included in the medium. In Expts. 8 and 9, 1.5M-KCl was added to a suspension of mitochondria in 0.25M-sucrose. (For details see text.)

Expt. no.	Internal water content of mitochondria (l./kg. of <i>M</i>)	Concens. of solute (millimolal)						Vol. of mitochondrial water non- accessible to sucrose (l./kg. of <i>M</i>)
		Sucrose		Potassium		Chloride		
		External	Internal	External	Internal	External	Internal	
1	1.58	264	200	1.2	24.0	0.1	7.0	0.38
2	1.53	302	236	1.9	22.8	0.1	6.2	0.33
3	1.44	361	254	1.2	26.9	0.1	7.0	0.43
4	1.33	496	354	1.0	31.3	0.1	8.4	0.38
5	1.54	264	199	23.8	54.4	22.9	20.9	0.37
6	1.44	259	191	47.7	78.7	46.9	35.4	0.38
7	1.34	248	177	111	133	113	85.0	0.42
8 <i>a</i>	1.63	250	132	0.9	52.8	0.1	5.9	0.77
8 <i>b</i>	1.38	241	157	48.4	106	49.6	44.6	0.48
9 <i>a</i>	1.70	247	137	0.4	61.0	0.1	8.9	0.76
9 <i>b</i>	1.33	250	126	39.8	104	42.4	28.0	0.66

Table 11. *Effect of a mixture of 0.25M-sucrose, 0.04M-sodium chloride and 0.04M-potassium chloride on the solute content of mitochondria*

Samples (2 ml.) of a suspension of mitochondria in 0.25M-sucrose were mixed with the appropriate volume of 3M-NaCl or 1.5M-KCl, or both. After standing for 10 min. at 0° the mitochondria were centrifuged for analysis.

Expt. no.	Total water content of pellet (l./kg. of <i>M</i>)	Concns. of solute (millimolal)							
		Sucrose		Sodium		Potassium		Chloride	
		S.*	P.*	S.	P.	S.	P.	S.	P.
1	2.54	263	182	0.2	0.4	0.3	42.5	0.1	4.3
2	2.34	244	182	41.6	36.1	0.2	41.7	39.3	33.3
3	2.34	254	200	0.4	0.4	41.6	83.0	39.4	32.6
4	2.31	235	199	43.3	35.8	40.3	79.8	85.1	65.5

* S., supernatant; P., pellet.

found in the pellet was almost exactly the sum of the endogenous potassium concentration found in Expt. 1 plus the concentration added to the medium, which is in agreement with the findings of Werkheiser & Bartley (1957). The distribution of sodium and chloride was, however, not the same as they reported. The added salts had no effect on total TCA-extractable phosphate content of the mitochondrial pellet, but in each case about 18% of the endogenous magnesium was lost from the mitochondria.

DISCUSSION

Water content of mitochondria. In this work the water content of the mitochondria, or alternatively of the whole mitochondrial pellet, was measured. This water content was taken for calculating the millimolal concentration of internal solutes, which was compared with the millimolal concentration of solutes in the supernatant.

Jackson & Pace (1956) did not estimate the water content of their preparations of mitochondria, but measured the total volume of the mitochondrial pellets. The total pellet volume was used for calculating the concentration of solutes in the pellet (as mg./ml. of pellet) which was compared with the concentration in the supernatant (as mg./ml. of solution). This method of expressing concentrations does not allow for the volume of dry matter in the mitochondrial pellet. It is therefore not possible to make quantitative comparisons of our results with those of Jackson & Pace.

Under their experimental conditions Jackson & Pace (1956) reported that the mitochondria soon swelled to several times their initial volume, particularly in saline media. This may perhaps have been due to their carrying out the incubations at 12.5°. In our work all incubations were at 0°, and very little swelling occurred.

Heterogeneity of mitochondrial preparations. The distribution of solutes among different fractions obtained during the isolation of mitochondria (Table 3) showed that until the final stage of the preparation a mixture of particles was present. Apparently some of the particles contained much potassium and phosphate and were fairly impermeable to sucrose, while other particles contained low concentrations of the endogenous solutes and were rather permeable to sucrose. The two kinds of particles were partially segregated, by the selection shown to occur during differential centrifuging or by means of their differing ability to penetrate silicone fluid of sp.gr. 1.055 on rapid centrifuging. The final preparation was not fractionated any further by spinning through silicone. However, it still contained enough sucrose to indicate that about one-tenth of the mitochondrial water was accessible

to this solute. This was an unusually low permeability to sucrose; in the majority of preparations about half to two-thirds of the mitochondrial volume was accessible to sucrose, with undiminished ability to penetrate silicone fluid on centrifuging.

The electron micrographs have shown that the preparations consisted only of mitochondria. They also showed that some of the mitochondria had a vesicular appearance, which was not seen in the mitochondria observed in sections of rat liver. Further, there was some correlation between the degree of vesiculation and the sucrose content. It is surmised that during the isolation procedure some of the mitochondria develop vesicles, lose endogenous potassium and phosphates, and become permeable to sucrose.

It is not known whether the above are 'all-or-nothing' phenomena as regards an individual mitochondrion. Nor could pure preparations of undamaged mitochondria be obtained, as judged by the criteria of solute content and permeability. Accordingly, in the treatment of the analytical data the results are interpreted in terms of accessible and non-accessible spaces rather than damaged or undamaged mitochondria.

Novikoff (1957) has published electron micrographs showing well-preserved isolated mitochondria and has contrasted them with the appearance of the particles found in the fluffy layer, which included much-swollen mitochondrial bodies. This is in accordance with our observation that, during the preparation, material was discarded which had a much higher degree of hydration than the final product.

Permeability of mitochondria. The mitochondria were partially, but never completely, permeable to sucrose. The volume of mitochondrial water which remained non-accessible to sucrose gradually decreased on prolonged storage of the mitochondria in 0.25 M-sucrose (Fig. 1). The results in Table 6 showed that, although the penetration of sucrose into the mitochondria was very slow, its removal by resuspending the mitochondria in a sucrose-free medium (0.125 M-potassium chloride) was very rapid. This behaviour is not in accordance with simple diffusion through a membrane, which, with a given concentration gradient, should proceed at an equal rate in either direction. It seems more likely that the penetration of sucrose is due to either breakdown or shrinkage of the barriers which initially confine the non-accessible space.

The experiments with 0.125 M-sodium chloride or -potassium chloride media (Tables 4 and 5) showed that the mitochondria were only partially permeable to Na⁺, K⁺ and Cl⁻ ions. The mitochondrial volume which appeared to remain non-accessible to these ions was approximately the same as that which was non-accessible to sucrose. However, it

was observed that on transfer of the mitochondria into the saline there were greater increases in the sodium or potassium concentration in the mitochondrial pellet than in its chloride concentration. It is considered that this discrepancy is due to an affinity of the mitochondria for certain cations. Bartley & Amore (1958) have shown that the discrepancy is much greater with manganese chloride, where the excess uptake of manganese at 0° apparently takes place by a process of cation-exchange adsorption. It may be recalled that transference of the mitochondria to a saline medium resulted in a small loss of magnesium from the particles. With concentrations of potassium up to about 50 millimolal the analyses indicated that the whole of the mitochondrial water was accessible to K⁺ ion (Tables 9–11; compare Werkheiser & Bartley, 1957). It is probable that under these conditions the existence of the non-accessible volume is masked by the cation affinity.

Mudge (1955) showed that the endogenous potassium of rabbit-liver mitochondria exchanged only very slowly with radioactive potassium added to the medium (containing 140 m-equiv./l. of potassium) in experiments carried out at 1°. Our results with rat-liver mitochondria were similar (Tables 5 and 8).

Jackson & Pace (1956) reported that the penetration of sucrose into the mitochondria had a half-time of a few hours, whereas isotopically labelled sodium and potassium entered very rapidly, with half-times of penetration of 10 min. or less. At the start of their experiments much higher rates of penetration were observed. The initial rapid entry is in accordance with our conclusion that a preparation of mitochondria contains two kinds of water space, one easily accessible to sucrose, sodium chloride or potassium chloride, and the other rather impermeable. However, the rates of penetration that they observed were much faster than were found in the present study. This may be related to their higher incubation temperature and the onset of swelling of the mitochondria.

Osmotic properties of mitochondria. It was shown (Table 10) that when mitochondria were exposed to hypertonic conditions there was a decrease in their water content. If Expts. 1 and 4 (Table 10) are compared, it is seen that when the concentration of sucrose in the medium was nearly doubled the shrinkage produced was only 16%. This was probably because 75% of the internal water of the mitochondria was penetrated by the sucrose, and accordingly this volume might be expected not to undergo shrinkage. Tedeschi & Harris (1955) also observed that isolated mitochondria did not undergo such large changes in total volume in different concentrations of sucrose as osmotic considerations would suggest. The discrepancy corre-

sponded with an osmotic dead-space of about 40–50% of the volume of the mitochondria in 0.25M-sucrose. They ascribed the dead space to the dry matter of the mitochondria, and concluded that the mitochondria were actually impermeable to sucrose. One of our preparations of mitochondria had 50% dry weight and was almost impermeable to sucrose, but it was exceptional.

The shrinkage produced by sucrose and by potassium chloride in equal osmolar quantities was equal (Table 10). This supported the analytical evidence that the permeability to both solutes was the same.

SUMMARY

1. The distribution of solutes between isolated rat-liver mitochondria and the suspension medium at 0° was studied by centrifuging and analysing the sedimented pellet and supernatant fluid.

2. Under standard conditions of centrifuging (26 000 g for 10 min.) the volume of extraparticulate fluid in the pellet was 24–59% of the total water of pellets sedimented from 0.25M-sucrose at 0°. The water content of the mitochondria was 1.0–1.8 l./kg. of dry weight.

3. Mitochondria isolated from 0.25M-sucrose contained sucrose at a concentration of 34–74% of that in the medium. They also contained 24–94 millimolal potassium. There was an inverse relationship between the sucrose and potassium content of different preparations of mitochondria.

4. During the isolation of mitochondria by differential centrifuging, dense particles containing a high concentration of potassium and little sucrose were partially separated from less dense particles with a low potassium content and much sucrose. Differing proportions of these particles in the final preparation accounted for the variable analyses of different mitochondrial preparations.

5. The sucrose was largely removed from isolated mitochondria by centrifuging and resuspending them in 0.125M-sodium chloride or 0.125M-potassium chloride.

6. A given preparation of mitochondria was initially about equally permeable to sucrose and to Na⁺, K⁺ and Cl[−] ions. There was a small excess uptake of sodium or potassium compared with chloride.

7. On prolonged storage of the mitochondria at 0° in 0.25M-sucrose or 0.125M-sodium chloride there was a gradual loss of endogenous potassium and phosphate, while some penetration by solutes of the medium occurred. In 0.125M-potassium chloride containing ⁴²K, the endogenous potassium of the mitochondria exchanged very slowly with potassium in the medium.

8. Hypertonic media caused a decrease in the water content of the mitochondria. The amount of

shrinkage indicated that about three-quarters of the mitochondrial water volume was osmotically dead space.

9. It is concluded that the mitochondrial preparations are probably mixtures of two kinds of particle. One kind has a low water content, is rich in potassium and phosphate and almost impermeable to sucrose, sodium chloride and potassium chloride. The other kind has a high water content and is rather permeable to these solutes.

10. Evidence from electron microscopy suggests that the former particles are relatively undamaged mitochondria, whereas the latter have developed a vesicular appearance.

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APPENDIX

A Lithium Internal-Standard Flame Photometer

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Berry, Chappell & Barnes (1946) showed that, in estimating sodium and potassium by flame photometry, there were definite improvements in precision and accuracy when lithium was added to the samples as an internal standard (compare Spencer, 1950; Bernstein, 1952). In this Appendix a simple coal-gas/air flame photometer is described which may be used either as a direct-reading instrument or with lithium internal standard.

The internal-standard flame photometer is a modification by two of us (J.E.A. and W.C.W.) of a direct-reading flame photometer, which was developed by one of us (D.S.P.) from the apparatus described by Leyton (1951). A commercial single-

cell direct-reading flame photometer based on the design of D.S.P. is available from A. Gallenkamp and Co., London, E.C. 2. This Appendix is limited to those essential particulars in which the internal-standard flame photometer differs from the Gallenkamp instrument.

EXPERIMENTAL

Description of the flame photometer

The stainless-steel concentric atomizer is fitted with a capillary tube of smaller bore (0.33 mm.) than is used in the Gallenkamp instrument, so that the rate of sample consumption is only 0.9–1.1 ml./min. with 9 lb./in.² air pressure. The aspiration rate may be decreased by fitting a finer hypodermic needle to the atomizer. The spray chamber and burner (Fig. 1) are quite different from those used by

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