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Solute Movements During Volume Changes in Rat-Liver Mitochondria

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The penetration of solutes into mitochondria has two separate aspects. In the first the fraction of the mitochondrial water that is penetrated by the solute under test at equilibrium is measured. The fraction of the mitochondrial water penetrated by the solute at the same concentration as that in the medium has been termed the 'space' of that solute (Amoore, 1958) and is measured by the ratio of the concentration of the solute in the medium to that in the mitochondrial water. Measurement of these 'spaces' (see Amoore & Bartley, 1958; Amoore, 1958) gives no indication of the relative rates at which different solutes penetrate, and a direct measurement of the time course of penetration of a solute is very difficult, since the distance the solute diffuses in penetrating the mitochondrion is very small (less than 1 μ) whereas the time required to isolate the mitochondria from the incubation medium is relatively long (10–15 min.). However, the composition of the fluid that enters or leaves the mitochondrion during the processes of swelling or shrinking must reflect the relative ease with which the individual solutes can pass through the membrane.

In this paper a detailed study is made of the composition of the fluid taken up by or expelled by the mitochondria when they swell or shrink. Most studies on mitochondrial swelling have been concerned only with the magnitude of the swelling and the conditions causing the swelling (e.g. the composition of the medium). Further, in most studies of swelling the increase in the volume of the mitochondria is some two or three times. With such a large increase in volume it is likely that the selectivity of the mitochondrial membrane to the passage of solutes will be greatly altered. Therefore in this work use has been made only of experiments in which the volume change of the mitochondria was relatively small.

MATERIALS AND METHODS

The preparation of rat-liver mitochondria, the incubation techniques, separation of mitochondria from the incubation medium and determination of the dry matter of the mitochondria were according to Werkheiser & Bartley (1957).

The following methods were used for the estimation of the various solutes: sucrose (Kulka, 1956); sodium and potassium, by the lithium internal-standard flame photometer (Amoore, Parsons & Werkheiser, 1958); phosphate, Berenblum & Chain (1938) as modified by Bartley (1953); chloride, Sanderson (1952); magnesium, Mann & Yoe (1956) as modified by Amoore & Bartley (1958); manganese, Bartley, Notton & Werkheiser (1957).

In the calculation of 'spaces' of the mitochondria the water of the centrifuged mitochondrial pellet was assumed to be equal to the mitochondrial water. No allowance was made for the extraparticulate water.

Experimental procedure. Mitochondria, prepared according to Werkheiser & Bartley (1957), were suspended in the experimental medium and maintained at the required temperature for the time stated in the various tables. At temperatures above 0° the mitochondria were shaken in air, usually in conical flasks. At the end of the incubation time measured samples of the mitochondrial suspension were added to accurately weighed centrifuge tubes (4–5 ml. capacity). After centrifuging for 5 min. at 25 000*g* the supernatant fluid was poured as quantitatively as possible into weighed conical centrifuge tubes containing 1 ml. of 30% trichloroacetic acid. The centrifuge tubes had been weighed after the addition of the trichloroacetic acid and hence the weight and volume of this fluid were accurately known. Duplicate samples of 1 ml. of the trichloroacetic acid differed by less than 1 mg. in weight. The contents of the centrifuge tubes were mixed and the tubes again weighed. Thus the weight of solution poured off from the mitochondrial pellet was accurately known. The weight of 1 ml. of the supernatant fluid was also determined and thus the volume of supernatant fluid was also known. The inside of the centrifuge tube containing the mitochondrial pellet was carefully dried with filter paper and the weight of pellet was determined. An accurately measured quantity of 10% trichloroacetic acid (1 ml.) was added to the mitochondrial pellet and the tube and contents were again weighed. The mitochondrial pellet was broken up with a glass rod, care being taken not to remove any particles of the precipitated protein, and the tube was set aside at 0° for 12 hr. to allow thorough extraction of the mitochondrial solutes. The centrifuge tubes and contents were centrifuged at 25 000*g* for 5 min. and the supernatant fluid was decanted for analysis. The solid residue in the centrifuge tube was stirred with 5% trichloroacetic acid (about 1 ml.) and again centrifuged. The supernatant from this procedure was discarded and the washing procedure was repeated. A final wash of the mitochondrial solids was made with water and 1 ml. of water was added to the pellet. The tube containing the pellet and water was then dried at 105°; the weight of the dried pellet was determined. The water content of the original mitochondrial pellet can be determined by subtracting the weight of the dried mitochondrial

residue together with the sum of the weights of the chemically determined solutes in the wet mitochondrial pellet from the weight of the wet mitochondrial pellet. The validity of this method of determining the water content of mitochondrial pellets has been established by Werkheiser & Bartley (1957). The mitochondrial extracts and the deproteinized suspension media were analysed for their various solute contents.

The analytical data thus available for mathematical manipulation are: (1) solid, solute and water content of the mitochondrial pellet; (2) solute content, weight, density and volume of suspension medium. From (2) the concentration of solutes in which the mitochondria were suspended can be calculated. In this case the concentrations can be expressed on a molal or a molar basis. From (1) the concentrations of mitochondrial solutes can be calculated but these values can only be expressed on a molal basis since the volume of original mitochondrial fluid is not known. However, it is known that mitochondria contain about 40% of soluble protein and thus a gross error would be introduced by equating molarity with molality in this context. The deviation from ideal behaviour of the solutions used in the preparation of mitochondria may not be generally realized. Thus 0·25 M-sucrose solution is commonly used with a tacit assumption that the osmotic pressure is 0·25 osmolal. But the volume of water contained in a 0·25 M solution of sucrose is only about 91·5% of the solution volume and the osmolality is thus not 0·25 but 0·27, a difference of some 8%. In this paper all the concentrations are expressed on a molal basis. Amounts of solutes are given as quantities associated with unit dry weight of mitochondria. These are expressed as m-moles/kg. of M, where M is the dry weight of the trichloroacetic acid-insoluble material of the mitochondria, which is within 1% of the dry weight of the mitochondria determined directly (see Werkheiser & Bartley, 1957).

Accuracy of the chemical determinations. Duplicate determinations agreed to better than $\pm 2\%$.

Method of calculation. From the measured amount of each solute in the mitochondrial pellets and the measured dry weight (M) of the mitochondrial pellet the amounts of each individual solute associated with 1 kg. of M were calculated. From the amount of water in each mitochondrial pellet the volume (l.) associated with 1 kg. of M was calculated. When the amount of water/kg. of M increased it was assumed that the mitochondria had swollen and when this value decreased then their volume had diminished. This assumption presumes that the partial specific volume of the dry solids is not materially altered by changes in the amount of water in the mitochondrial pellet. By comparing the amounts of solutes and water associated with unit weight of mitochondrial solids at different times an assessment can be made of changes of water and solutes occurring in the mitochondrial pellets between the two times. The changes in solute and water may be independent. For example, the solute in the mitochondrial pellet may increase without a change in the water content. With sucrose this is assumed to be due to penetration of the solute into part of the water space hitherto inaccessible. On the other hand, the water content of the mitochondria may change without a change in the total solute content of the mitochondria. Usually there is a simultaneous change in both water and solute content of mitochondria.

RESULTS

Solute movements during mitochondrial-volume changes in sucrose solutions. Mitochondria do not swell appreciably in 0·25 M-sucrose at 0°, although they become increasingly permeated with sucrose (see Amoore & Bartley, 1958). However, swelling may occur in this medium when the temperature is raised to 20° and shrinking may be induced at 0° by transfer of the mitochondria from 0·25 M-sucrose to sucrose solutions of a higher osmotic pressure. In Table 1 is shown the composition of the fluid passing into the mitochondria, compared with the composition of the suspending medium, when swelling was induced by keeping at 20° for 1 hr. The amount of sucrose that penetrated the mitochondria was greater than that contained in the volume of medium that entered the mitochondria. Thus the proportion of the mitochondrial pellet permeated by sucrose increased. The data suggest that two processes occur: a penetration of sucrose alone into the mitochondrial pellet because of diffusion of this solute down the concentration gradient, and bulk movement of sucrose solution into the mitochondria. Under these conditions the water and the sucrose move in the same direction. By placing mitochondria suspended in 0·25 M-sucrose into sucrose solutions of a higher or lower osmotic pressure it is possible to induce a movement of water in the opposite direction from the net sucrose movement (Table 1, Expts. 2 and 3). Swelling or shrinking induced by transfer to solutions of different osmotic pressure increased the loss of endogenous potassium. Shrinkage by transfer to a sucrose medium of high osmotic pressure did not alter the volume of sucrose solution in the pellet that was at the same concentration as in the medium. That is, the shrinkage was extrusion of fluid from that part of the mitochondrial pellet into which the sucrose had not penetrated. It can be seen from Table 1 that all the potassium content of the pellet and an equivalent amount of anion must be allotted to the sucrose-free water of the pellet to obtain approximately iso-osmolality with the medium. On resuspending the 'hyperosmotic' mitochondria in 0·25 M-sucrose the uptake of water was greater than that lost by the previous shrinkage, and the volume occupied by iso-osmolal sucrose increased by 45% whereas the sucrose-free water increased by about 15%. Again, all the potassium must be present in the sucrose-free water of the mitochondria for this to have the same osmotic pressure as the medium. The loss in K^+ ions that occurred on transfer from solutions of higher osmolality to 0·25 M-sucrose is as would be predicted if all the potassium was contained in the sucrose-free water. The reciprocal relationship

Table 1. *Movement of water and solutes with volume changes of mitochondria suspended in sucrose solutions*

The total quantity of each solute, the water content and the dry matter of the mitochondrial pellets were determined as described in the text. Duplicates of chemical determinations agreed better than ±2%. Wet mitochondrial pellets weighed 100–150 mg. and the dry matter was 30–50 mg. Duplicate weighings agreed within 0·2 mg. Concentrations are calculated from the measured quantities of solutes and water by determining how much of each solute was contained in 1 kg. of water. The amount of solute taken up or extruded is calculated from the chemically determined amounts in the pellets. The composition of the solution taken up or extruded is calculated from the differences in the determined solute and water contents of the two mitochondrial pellets. The sucrose-containing water is calculated from the sucrose content of the mitochondrial pellet as that fraction of the pellet water necessary to contain the pellet sucrose at the same concentration as in the medium.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water			Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Kept at 20° for 60 min.	1·75	0·46	2·2	0·39	K^+	−3·1	35·2	—	2·6
						Cl^-	7·7	1·76	20	1·6
						Sucrose	108	191	285	241
						Mg^{2+}	−1·7	18	—	—
2	Suspension prepared in 0·25 M-sucrose then sufficient 2·5 M-sucrose was added to make the final concn. of sucrose about 0·5 M. Kept at 0° for 15 min.	1·4	0·64	1·35	0·27	K^+	−13	41·4	31	0
						Cl^-	−1·29	0·63	3	0
						Sucrose	336	179	—	541
3	Mitochondrial pellet in approx. 0·5 M-sucrose resuspended in approx. 0·25 M-sucrose; kept for 15 min. at 0°	1·35	0·27	1·96	0·31	K^+	−29·2	44·1	—	0
						Cl^-	0	0	—	0
						Sucrose	−200	433	—	281

Table 2. *Movement of water and solutes with volume changes of mitochondria suspended in sucrose solution containing small amounts of potassium chloride*

For method of calculation see Table 1 and text.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water			Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Kept in 0·25 M-sucrose + 0·01 M-KCl for 15 min. at 0°	2·18	0·65	2·6	0·43	K^+	24·8	26·5	124	12·5
						Cl^-	23·9	4·7	119	10·3
						Sucrose	64	197	320	240
						Mg^{2+}	−2·4	11·4	—	—

Table 3. *Movement of water and solutes with volume changes of mitochondria suspended in sucrose solutions containing small amounts of magnesium chloride*

For method of calculation see Table 1 and text.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water			Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Kept in 0·25 M-sucrose + 2 mM-$MgCl_2$ for 15 min. at 0°	2·18	0·65	2·16	0·55	K^+	−19	26·5	146	2·6
						Cl^-	9·4	4·7	—	5·2
						Sucrose	2·0	197	—	260
						Mg^{2+}	12·8	11·4	—	1·9
2	Kept in 0·25 M-sucrose + 5 mM-$MgCl_2$ for 15 min. at 0°	2·18	0·65	2·02	0·51	K^+	−18·9	26·5	63	2·5
						Cl^-	27·9	4·7	—	13·8
						Sucrose	−56	197	186	248
						Mg^{2+}	27·6	11·4	—	6·4

between sucrose and potassium content of mitochondrial preparations isolated in sucrose solutions has already been pointed out by Amoore & Bartley (1958).

Swelling or shrinking was also induced in mitochondria suspended in 0·25 M-sucrose solution at 0° by the addition of comparatively small amounts of salts (Tables 2 and 3).

It is not clear why swelling should occur when small amounts of KCl are added to the medium. Amoore & Bartley (1958) have shown that KCl, added to a mitochondrial suspension in sucrose solution, rapidly equilibrates with the sucrose-containing fluid of the mitochondrial pellet. This is demonstrated in this experiment by the large increase in the potassium content of the mitochondrial pellet. The sucrose-containing water of the pellet increased from 2·18 to 2·6 l./kg. of *M* whereas the sucrose-free water diminished from 0·65 to 0·43 l./kg. of *M*. This type of swelling should be contrasted with the mitochondrial shrinkage that occurred in sucrose solution of high osmolality, where all the loss in water was contributed by the sucrose-free fluid.

Shrinkage in the presence of magnesium chloride. Mitochondria shrank slightly when approx. 2 mM-$MgCl_2$ was added to a suspension in 0·25 M-sucrose solution. The diminution in volume was almost all due to a loss in the sucrose-free water. However, when the $MgCl_2$ added was 5 mM there was a loss of both the sucrose-containing and the sucrose-free water. With both magnesium concentrations the loss of potassium was approximately equal to the potassium content of the sucrose-free water lost (Table 3). It is assumed here that all the endogenous potassium is contained in the sucrose-free water of the pellet. In contrast, the potassium lost by the mitochondria, when shrinkage due to exposure to hyperosmotic sucrose solutions occurred, was only about 30% of that expected by the loss of the sucrose-free water.

Shrinkage in the presence of manganese chloride. Manganese ions also produced a shrinkage of mitochondria but the effect was much larger than with magnesium (Table 4). Thus 1 mM-Mn^{2+} ions produced about the same shrinkage as 2 mM-Mg^{2+} ions. With this low concentration of Mn^{2+} ions the shrinkage was largely in the sucrose-free water, but with higher concentrations both sucrose-containing and sucrose-free water decreased. The potassium loss was constant and independent of the manganese concentration added. It was about twice that expected by the shrinkage of the sucrose-free water.

Shrinkage in the presence of hydrochloric acid. As with $MnCl_2$ and $MgCl_2$ the addition of HCl (0·5 mM) caused a diminution in the sucrose-free water of the pellet, and as with these two salts the

Table 4. *Movement of water and solutes with volume changes of mitochondria suspended in sucrose solutions containing small amounts of manganous chloride*

For method of calculation see Table 1 and text.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water			Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Kept in 0·25 M-sucrose + 1 mM-$MnCl_2$ for 15 min. at 0°	2·18	0·65	2·14	0·52	K^+	−32·3	26·5	190	2·9
						Cl^-	−0·3	4·7	2	2·0
						Sucrose	−10·0	197	59	257
						Mg^{2+}	−6·3	11·4	37	—
						Mn^{2+}	25·6	—	—	0·1
2	Kept in 0·25 M-sucrose + 2 mM-$MnCl_2$ for 15 min. at 0°	2·18	0·65	1·82	0·49	K^+	−31·7	26·5	61	2·8
						Cl^-	4·2	4·7	—	4·1
						Sucrose	−92	197	176	256
						Mg^{2+}	−4	11·4	18	—
						Mn^{2+}	43	—	—	0·55
3	Kept in 0·25 M-sucrose + 5 mM-$MnCl_2$ for 15 min. at 0°	2·18	0·65	1·73	0·47	K^+	−32·8	26·5	52	2·8
						Cl^-	20·6	4·7	—	10·4
						Sucrose	−128	197	203	250
						Mg^{2+}	−11·3	11·4	18	—
						Mn^{2+}	60	—	—	2·3

Table 5. *Movement of water and solutes with volume changes of mitochondria suspended in sucrose solutions containing small amounts of hydrochloric acid*

For method of calculation see Table 1 and text.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water			Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Kept in 0·25 M-sucrose + 0·5 mM-HCl for 15 min. at 0°	2·18	0·65	2·28	0·4	K^+	−36·6	26·5	244	2·4
						Cl^-	3·8	4·74	25	0·4
						Sucrose	17	197	—	253
						Mg^{2+}	−3·6	11·4	24	—
2	Kept in 0·25 M-sucrose + 1 mM-HCl for 15 min. at 0°	2·18	0·65	2·31	0·39	K^+	−42	26·5	323	2·7
						Cl^-	−4·3	4·7	33·8	0·7
						Sucrose	37	197	—	258
						Mg^{2+}	−3·3	11·4	25·4	—
3	Kept in 0·25 M-sucrose + 2 mM-HCl for 15 min. at 0°	2·18	0·65	2·04	0·41	K^+	−46	26·5	121	2·7
						Cl^-	0·7	4·7	—	1·2
						Sucrose	−35	197	92	258
						Mg^{2+}	−6	11·4	15·9	—

Table 6. *Movement of water and solute with volume changes of mitochondria suspended in a mixture of sucrose and potassium chloride solution*

'Treatment of mitochondria' describes the history of the mitochondrial preparation up to the 'initial time' (at this time a sample of the mitochondrial suspension was removed and the mitochondria were sedimented for analysis: the analyses are given under 'Initial water content of mitochondrial pellet' and under 'Concn. of solute', 'Initial water of pellet') and its subsequent treatment during the experimental period. For method of calculation see Table 1 and text.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water			Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Mitochondria prepared in 0·25 M-sucrose were added to KCl soln. to give suspension medium containing 0·1 M-KCl and 0·05 M-sucrose (initial time) then kept at 0° for 60 min.	1·74	0·65	1·81	0·69	K^+	23	106	209	108
						Sucrose	3·6	36·3	32·7	49·9
2	As above but warmed to 21° for 5 min.	1·74	0·65	1·80	0·75	K^+	17·2	106	169	108
						Sucrose	8·0	36·3	19·4	49·9
3	As above but warmed to 21° for 25 min.	1·74	0·65	2·41	0·74	K^+	133	106	175	108
						Sucrose	33·1	36·3	43·5	49·9
4	Mitochondria prepared in sucrose solution were added to KCl soln. to give approx. 0·1 M-KCl and 0·05 M-sucrose in suspending medium. Kept for 5 min. at 0° (initial time) then maintained for further 55 min. at 0°	3·89	0	4·36	0	K^+	28	124	59·5	115
						Cl^-	136	109	290	113
						Sucrose	23	50	49	49·7
						Mg^{2+}	−5·5	3·7	—	0·25
						PO_4^{3-}	−24·1	19·3	—	0·6
5	Mitochondria prepared in sucrose soln. as above but maintained for 35 min. at 0° (initial time) then kept at 23·8° for 22·5 min.	4·20	0	6·11	0·91	K^+	250	119	119	110
						Cl^-	272	115	130	108
						Sucrose	96	50	45·5	50
						Mg^{2+}	1·2	1·9	0·57	0·26
						PO_4^{3-}	−8·1	12·5	—	0·71
6	Mitochondria prepared in 0·25 M-sucrose with added KCl to give final concn. 0·04 M (initial time) were then incubated for 60 min. at 20°	2·11	0·54	3·87	0	K^+	1	80·5	0·79	38
						Cl^-	78·9	33·1	62	42
						Sucrose	439	179	345	232

Table 7. *Movement of water and solutes with volume changes of mitochondria suspended in potassium chloride solutions*

For method of calculation see Table 1 and text. For other details see Table 6.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water			Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Prepared in 0·25 M-sucrose, suspended in 0·125 M-KCl at 0° (initial time) and then kept at 0° for 15 min.	1·37	0·67	2·61	0	K^+	267·4	41·4	46·9	127
						Cl^-	267·4	0·63	461	125
						Sucrose	−326·9	179	—	12·4
2	Prepared in 0·25 M-sucrose, suspended in 0·125 M-KCl at 0° (initial time) and then kept at 0° for 8 hr.	2·36	0·18	2·61	0·44	K^+	93	122	182	122
						Cl^-	106	68·5	207	119
						Sucrose	3·1	8·2	6·1	9·0
						PO_4^{3-}	−20·6	37·1	—	—
						Mg^{2+}	−4·0	9·2	—	—
3	Prepared in 0·25 M-sucrose, sucrose soln. (2·5 M) added to suspension to make sucrose concn. 0·5 M; centrifuged mitochondria (initial time) and resuspended pellet in 0·125 M-KCl was kept at 0° for 15 min.	1·29	0·33	3·02	0·25	K^+	358	44·1	217	127
						Cl^-	325	0	213	125
						Sucrose	−646	433	—	18·6
4	Prepared in 0·25 M-sucrose, KCl soln. (1·25 M) added to suspension to make KCl concn. 0·125 M (initial time) and resuspended pellet in 0·125 M-KCl was kept at 0° for 15 min.	1·32	0·45	2·69	0	K^+	53	174	59	127
						Cl^-	51	120	57	131
						Sucrose	−290	179	—	6·8
5	Prepared in 0·25 M-sucrose, sucrose soln. (2·5 M) added to made concn. 0·5 M, centrifuged and resuspended in 0·125 M-KCl (initial time) and then kept at 0° for 15 min.	1·78	0·49	7·0	0	K^+	858	18·7	181	129
						Cl^-	885	0	187	128
						Sucrose	−435	221	—	8·9
6	Prepared in 0·25 M-sucrose, KCl soln. (1·25 M) added to make 0·125 M-KCl, centrifuged (initial time), suspended in 0·125 M-KCl and then at 0° for 15 min.	1·49	0·53	6·9	0·42	K^+	840	44·7	159	129
						Cl^-	909	0	171	124
						Sucrose	−350	198	—	7·7

'sucrose space' was little affected by further increase in the HCl content of the medium (Table 5). The sucrose-containing water was not diminished by the lower concentration of HCl (cf. Tables 3 and 4 with Mg^{2+} and Mn^{2+} ions); instead some slight increase occurred. The higher concentrations produced a contraction of the sucrose-containing water similar to that which occurred with $MnCl_2$ and $MgCl_2$.

Volume changes of mitochondria in sucrose–potassium chloride mixtures. Usually storage at 0° or at room temperature in a solution containing sucrose and KCl of 0·25 osmolal caused a swelling. The magnitude of this varied (compare Expts. 1–3 with Expts. 4–6, Table 6). When the water content of the mitochondria reached about double that of normal mitochondria isolated in 0·25 M-sucrose (about 2·0 l. of water/kg. of *M*), they became totally permeable to sucrose. It was only in exceptional circumstances that the fluid taken up by the mitochondria corresponded in composition with the suspending medium (e.g. Expt. 5, Table 6). This swelling, which corresponds with that described by Leaf (1956), occurred when the water content of the mitochondria exceeded 4·0 l./kg. of *M* and when the sucrose-containing water occupied the whole of the mitochondrial water. Below this degree of swelling the mitochondria retained some degree of selectivity towards the composition of the fluid passing into or out of them. For example, in Expt. 4, Table 6, there is a marked discrepancy in the amount of anion and cation taken up by the mitochondria. It is not at all clear, for example, what is the compensating ion making for electrical neutrality in this experiment. The pH of the internal fluid of the mitochondria is not known but it seems unlikely that it would differ greatly from neutrality. There must therefore be a lowering of the pH of the internal fluid of the mitochondria, the magnitude of which will depend on the buffering capacity of the internal proteins. The uptake of chloride was partly compensated for by loss of phosphate, but there remained the equivalent of some 70 m-moles of chloride to be compensated for. This in unbuffered solution would lower the pH to between 1 and 2. Experiments in which the mitochondria were directly titrated with HCl (Amoore & Bartley, 1958; Bartley & Amoore, 1958) showed that the mitochondria will buffer so that the addition of 1 μmole of HCl to 27 mg. of mitochondrial dry matter in 1 ml. produced a pH drop of only 0·3 unit. This absorption of H^+ ions may be a reversal of the acid secretion which occurred when $MnCl_2$ was added to the mitochondrial suspensions.

Volume and solute changes of mitochondria suspended in potassium chloride solutions. In these experiments the mitochondria were prepared in

Table 8. *Movement of water and solutes with volume changes of mitochondria suspended in various saline media*

For method of calculation see Table 1 and text.

Expt. no.	Treatment of mitochondria	Initial water content of mitochondrial pellet (l./kg. of *M*)		Final water content of mitochondrial pellet (l./kg. of *M*)		Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
								Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	Prepared in 0·25 M-sucrose and suspended in a medium containing 24·2 mM-K^+, 6·5 mM-Na^+, 22 mM-Cl^-, 4 mM-PO_4^{3-}, 5 mM-α-oxoglutarate, 5 mM-AMP; incubated for 10 min. at 25°	2·93		3·63		Na^+	4·1	14·6	5·9	8·8
						K^+	−3·0	61·5	—	29·6
2	Prepared in 0·25 M-sucrose and then suspended in medium at 0° for 17 min. (concentrations of solutes in medium are given under 'Supernatant water')	2·68		3·29		K^+	30	99	49·2	88
						Na^+	−4·3	30·4	—	27·5
		Sucrose-containing water	Sucrose-free water	Sucrose-containing water	Sucrose-free water	Cl^-	21	63·1	34·5	67·0
						PO_4^{3-}	12·5	39·4	20·5	18·5
						Sucrose	20	43·7	32·8	46·4
		2·52	0·16	2·96	0·33	Mg^{2+}	1	7·2	1·6	5·0

0·25 M-sucrose solution and after various treatments were suspended in 0·125 M-KCl. Since the particles were not washed with the KCl solution a small amount of sucrose (6–12 m-molal) remained in the suspending fluid. This enabled an estimate to be made of the sucrose-containing and sucrose-free water. Resuspension of the mitochondria in 0·125 M-KCl at 0° (Expt. 1, Table 7) caused a 28% increase in the water content and rendered the pellet completely permeable to sucrose. The amounts of K^+ and Cl^- ions passing into the pellet showed that these could freely diffuse throughout the water of the pellet and their uptake was limited to the amount contained in the volume of solution that entered the mitochondria. In some experiments (Table 7) the impermeability to sucrose was not completely abolished by transfer to 0·125 M-KCl solution. As the preparation swelled when stored at 0° there was an increase in volume of both the sucrose and sucrose-free water. The chloride, which was taken up in greater amount than K^+ ions, was now evenly distributed throughout the pellet water, but there was a net overall excess of K^+ ions, resulting in the water of the pellet having a K^+ ion concentration of 142 m-molal. It seems unlikely that all the K^+ ions can be in free solution in the pellet water. It is possible that the swelling of the mitochondria resulted in an unfolding of protein structures with the formation of new cation-binding sites on the protein. It appears from Expts. 3 and 4 (Table 7) that the rate of movement of water must be faster than that of sucrose and the mitochondrial membrane must retain some semipermeable properties, since swelling of the sucrose space occurred in both these experiments. Apparently the treatment given to the mitochondria described in Expts. 5 and 7 (Table 7) greatly increased the permeability of the mitochondria to KCl and water without greatly altering that to sucrose. This resulted in the swelling shown in these two experiments.

Movement of water and solutes with volume changes of mitochondria in saline media. Mitochondria prepared in 0·25 M-sucrose solution and suspended in the medium shown in Table 8 (Expt. no. 2) swelled at 0°. Both the sucrose and the sucrose-free water increased but the percentage increase was greater in the sucrose-free water. It can be seen that the composition of the fluid taken up by the mitochondria is very different from that of the incubation medium, showing that the mitochondrial membrane still retains its differential permeability.

Effect of calcium and magnesium on sodium, potassium and water movement in mitochondria. In these experiments the mitochondria were suspended in the type of medium in which maximum rates of uptake of O_2 are expected to occur. The mitochondria were added to the medium at 0° and subsequently warmed to 25°. As shown in Table 9, calcium and magnesium had opposite effects on the initial potassium content of the pellet. Calcium caused a loss of potassium from the mitochondria, whereas magnesium caused the endogenous potassium to be retained better than in its absence. Both calcium and magnesium caused a slight swelling of the mitochondrial pellet during the initial period at 0° but on subsequent incubation at 25° magnesium prevented the swelling that occurred without magnesium or in the presence of calcium. Magnesium also lessened the uptake of sodium from the medium at 0°, whereas calcium had little effect. When incubated at 25° the opposite roles of calcium and magnesium again became apparent since the presence of calcium increased the uptake of sodium, whereas the presence of magnesium decreased it. The uptake of sodium in all three cases was against a concentration gradient but the nominal concentration of sodium in the solution that was taken up in the three cases was highest with magnesium and lowest in the absence of both calcium and magnesium. Similar observations

Table 9. *Effect of calcium and magnesium on solute and water movement in mitochondria*

For method of calculation see Table 1 and text. The mitochondria were prepared in sucrose and suspended in a medium containing 24·2 mM-K^+ ions, 22 mM-Cl^- ions, 3 mM-PO_4^{3-} ions, 5 mM-α-oxoglutarate and 5 mM-AMP; in Expt. 2 a further addition of 2·5 mM-Ca^{2+} ions was made and in Expt. 3 2·5 mM-Mg^{2+} ions. Incubation was for 10 min. at 25°.

Expt. no.	Initial water content of mitochondrial pellet (l./kg. of *M*)	Final water content of mitochondrial pellet (l./kg. of *M*)	Solute	Solute taken up or extruded (m-moles/kg. of *M*)	Concn. of solute (m-molal)		
					Initial water of pellet	Soln. extruded or taken up	Supernatant water
1	2·93	3·63	Na^+	4·1	14·6	5·9	8·8
			K^+	−30	61·5	—	29·6
2	3·04	3·60	Na^+	7·3	12·9	13	8·8
			K^+	0	46·4	0	29·6
3	3·03	3·17	Na^+	2·5	9·7	17·8	8·8
			K^+	−7	65·7	—	29·6

have been made on the permeability of red-cell 'ghosts' by J. F. Hoffman, D. C. Tosterson & R. Whittam (unpublished work).

DISCUSSION

Leaf (1956) has shown that under certain conditions the fluid entering the tissues in the process of swelling was a solution approximately iso-osmotic with the medium. The distribution of the imbibed fluid within the cell is not known, but it is reasonable to suppose that it will not be confined to the extramitochondrial fraction of the cell. If the fluid taken up is evenly distributed throughout the cell volume, then the intracellular mitochondria cannot be exhibiting the same permeability characteristics as the isolated mitochondria since these swelled only with uptake of unaltered medium, when they had been subjected to gross osmotic damage. Yet it seems unlikely that the permeability of the isolated mitochondria would be less than when they were in the cell. On the other hand, if the swelling described by Leaf (1956) does not involve the mitochondrial compartment of the cell then no deductions can be made about the state of the mitochondrial membrane within the cell.

The passive passage of a solute through a membrane is dependent on the driving force supplied by the differences in the external and internal concentrations. The ease with which this transfer of solute takes place is dependent on the permeability of the membrane to the particular solute. Thus if the volume of the mitochondria is increased we should expect solutes to pass into the mitochondria in the same proportion as in the medium if the mitochondrial membrane is freely permeable to all these solutes. As seen by the data presented, this is seldom realized. The ratio of concentration of solute in water taken up/concentration of solute in the medium is a measure of the permeability of the mitochondrial membrane to the solute. This value will be 1 if the membrane is freely permeable to the solute and 0 if the membrane is completely impermeable to the solute. It is important to realize that this ratio is independent of the absolute concentration of solute in the medium. The value can be expressed as a percentage of the water permeability by multiplying the values by 100. A similar argument can be applied to the shrinkage of mitochondria where concentration of solute in water extruded/concentration of water in mitochondrial medium measures the permeability of the membrane to the internal solutes. If the value for the permeability is above 100 this suggests that either some form of absorption is occurring, and all the solute passing into the mitochondria is not in solution, or the passage of the solute has been facilitated by some metabolic process. It is clear that the estimate of the permeability is liable to be too low (*a*) when there is an active extrusion process for any solute working in the opposite direction to the bulk solute flow, and (*b*) when there is a concentration gradient of the solute in the opposite direction to the bulk solute flow. When for any reason the net solute movement is in the opposite direction to the bulk solution flow the solute movement is given a negative sign. Any results where (*a*) or (*b*) can be shown to occur or where the sign of the solute movement is negative have not been considered in the assessment of the permeability of the mitochondrial membrane. From Table 10 it can be seen that the permeability of the mitochondria to sucrose is variable. Usually sucrose does not penetrate the mitochondrial membrane as readily as does water, the mean percentage permeability being 78 %. The passage of sucrose through the mitochondrial membrane does not render the membrane freely permeable to sucrose, as shown by the comparatively small amount of this solute extruded on shrinkage. The

Table 10. *Comparison of sucrose and water passage through the mitochondrial membrane*

The passage of water is taken as 100 %; the values of the sucrose movement are considered to be the same as the water movement if the concentration in the solution taken up or extruded is the same as that in extramitochondrial fluid or intramitochondrial fluid respectively.

Expt. no.	Experimental conditions	Permeability (%) compared with water	Volume change of mitochondria (%)
1	20°; 60 min. in 0·25 M-sucrose	118	+17
2	0°; 15 min. in 0·25 M-sucrose + 0·01 M-KCl	133	+7
3	0°; 15 min. in 0·25 M-sucrose + 5 mM-$MgCl_2$	95	−10·5
4	0°; 15 min. in 0·25 M-sucrose + 1 mM-$MnCl_2$	30	−6
5	0°; 15 min. in 0·25 M-sucrose + 2 mM-$MnCl_2$	90	−18
6	0°; 15 min. in 0·25 M-sucrose + 5 mM-$MnCl_2$	103	−22
7	0°; 15 min. in 0·25 M-sucrose + 2 mM-HCl	46·5	−14
8	0°; 60 min. in 0·05 M-sucrose + 0·1 M-KCl	65	+5
9	21°; 5 min. in 0·05 M-sucrose + 0·1 M-KCl	100	+7
10	21°; 25 min. in 0·05 M-sucrose + 0·1 M-KCl	87	+31·5
11	0°; 17 min. in medium described in Table 8 (Expt. no. 2)	71	+23

results of Expts. 8 and 9 (Table 10) suggest that the permeability of the mitochondrial membrane to sucrose is diminished by increase in temperature. The effect of increasing manganese concentration increases the shrinkage of the mitochondria and increases the permeability of the mitochondria to sucrose. In most of the experiments given in Table 10 the movement of potassium was also measured. These results (Table 11) show that potassium permeability was uniformly larger than 100%. Since most of these experiments were carried out at 0° it seems that these high values are the results of the participation of adsorption processes during the exchange of potassium between mitochondria and medium. Adsorption of potassium has already been demonstrated by Amoore & Bartley (1958). In the one experiment (Expt. 10, Table 11) where relative impermeability to potassium could be demonstrated, the percentage permeability was less than that of sucrose and very similar to that of chloride (see also Amoore, 1958).

The percentage permeability of sodium is generally similar to that of sucrose and chloride (Amoore & Bartley, 1958; Bartley & Amoore, 1958), but this can vary with the presence of bivalent cations in the medium. In a medium suitable for oxidative phosphorylation (Table 9) the percentage permeability of sodium was 67, but on the addition of 2·5 mM-calcium it rose to 148 and with 2·5 mM-magnesium it became 202. It seems probable that in this case the effect of the bivalent cations is to liberate binding sites suitable for the attachment of sodium.

All these experiments illustrate the variation in the properties of adsorption and permeability of the mitochondrial membrane that can result from changes in the composition of the suspending medium. It is to be supposed that changes in the metabolic properties of the mitochondria will follow from these variations. It would seem particularly important when studies are being made on mitochondrial swelling to give a full description of the water and solute content of the starting material, since the degree of swelling or shrinking will largely depend on this. This information is very seldom given and the situation is further complicated by the frequent use of optical methods to measure swelling, which are usually not calibrated against the changes in the water content of the mitochondria and which ignore the possible contributions to the changes in light-scattering of the changes in refractive index of the intramitochondrial solution. Tedeschi & Harris (1955), from light-scattering studies, had concluded that mitochondria were impermeable to sucrose and behaved as osmometers in solutions of this solute. The work of Werkheiser & Bartley (1957) and Amoore & Bartley (1958) showed by direct measurement that sucrose did in fact penetrate liver mitochondria to a variable extent, usually some 50–60% of their volume. Recknagel & Malamed (1958), who first doubted the validity of the observations of Werkheiser & Bartley (1957), have since confirmed them (Malamed & Recknagel, 1959). They conclude that the fraction of the mitochondrial volume into which sucrose does not penetrate responds as expected by volume changes when the concentration of sucrose in the suspending medium is varied. This fact was already apparent in the results of Werkheiser & Bartley (1957) and is demonstrated in this paper. However, it is not only the sucrose-free space of the mitochondria that changes in volume in response to changes in the external medium. Bivalent cations may cause shrinkage of the sucrose-containing water as well as the sucrose-free space and, in mitochondria which have been penetrated completely by sucrose, volume changes occur in response to changes in the medium. It is still not clear what significance the changes in mitochondrial permeability *in vitro* have for the understanding of the behaviour of mitochondria *in vivo*. It is an attractive theory that permeability changes may be linked with the control of the rate and direction of cellular metabolism. There is as yet no evidence contradicting this supposition but the possibility must also be

Table 11. *Comparison of passage of potassium chloride and water through the mitochondrial membrane*

Expt. no.	Experimental conditions	K permeability (%) compared with water	Cl permeability (%) compared with water	Volume change of mitochondria (%)
1	0°; 15 min. in 0·25 M-sucrose + 0·01 M-KCl	1000	1000	+7
2	0°; 15 min. in 0·25 M-sucrose + 5 mM-$MgCl_2$	237	—	−9·5
3	0°; 15 min. in 0·25 M-sucrose + 1 mM-$MnCl_2$	720	—	−6
4	0°; 15 min. in 0·25 M-sucrose + 2 mM-$MnCl_2$	230	—	−18
5	0°; 15 min. in 0·25 M-sucrose + 5 mM-$MnCl_2$	196	—	−22
6	0°; 15 min. in 0·25 M-sucrose + 2 mM-HCl	456	—	−14
7	0°; 60 min. in 0·05 M-sucrose + 0·1 M-KCl	193	—	+5
8	21°; 5 min. in 0·05 M-sucrose + 0·1 M-KCl	156	—	+7
9	25°; 5 min. in 0·05 M-sucrose + 0·1 M-KCl	162	—	+31·5
10	0°; 15 min. in medium described in Table 8 (Expt. no. 2)	55·9	51·4	+27

considered that the permeability characteristics merely reflect the chemical structure of the membrane and are not in themselves of importance as mechanisms regulating metabolism.

SUMMARY

1. The water and solute content of rat-liver mitochondria have been measured during swelling or shrinking of the particles.

2. When mitochondria swell in 0·25M-sucrose solution there is an increase in the fraction of mitochondrial water penetrated by sucrose. The shrinkage due to transfer of mitochondria from 0·25M-sucrose to solutions of a higher osmoticity is mainly due to the loss of water from the sucrose-free space.

3. In mitochondria partially permeated by sucrose it is necessary to assume for osmotic equilibrium that the endogenous potassium is solely within a compartment separate from the sucrose.

4. When magnesium or manganese cause a shrinkage of mitochondria there is a loss of potassium that is never greater than that expected from the volume change of the sucrose-free water.

5. When mitochondria have swollen to a state when they contain more than 4 l. of water/kg. of solids they become completely permeable to sucrose and in further swelling the volume changes are due to an uptake of the suspending medium. Below 4 l. of water/kg. of solids the mitochondria are able partially to exclude some components of the medium.

6. Calcium and magnesium have antagonistic effects on mitochondria. Calcium increases the uptake of sodium and increases the loss of potassium, whereas magnesium decreases both these processes.

7. The problems of mitochondrial permeability are discussed.

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Selective Determination of Sugars Manifesting Enediol Isomerism by means of Reaction with Tetrazolium

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2:3:5-Triphenyltetrazolium chloride in aqueous alkaline solution (Wallenfels, 1950) has been used as a selective spray reagent on paper for the detection of sugars manifesting 1:2-enediol isomerism (Bell & Dedonder, 1954; Feingold, Avigad & Hestrin, 1956; cf. also Barker, Bourne, Grant & Stacey, 1956; Haq & Whelan, 1956; Côté, 1959; O'Donnell & Percival, 1959). It has been noted by Bacon (1959) that the reaction of 2:5-diphenyl-3-(4-styrylphenyl)tetrazolium chloride yields a deeper colour than does the reaction with triphenyltetrazolium chloride. In the present paper a selective quantitative assay of sugars, manifesting enediol isomerism, is based on reaction with these tetrazolium reagents and is used in particular for the selective determination of oligofructosides bearing a substituent on C-6 (compounds of the type of 6-R-fructose) in the presence of those bearing a substituent on C-1 (compounds of the type of 1-R-fructose).