BBA 75022

WATER EXTRUSION IN ISOLATED SUBCELLULAR FRACTIONS VI. OSMOTIC PROPERTIES OF SWOLLEN PHOSPHOLIPID SUSPENSIONS

RENZO RENDI
Departments of Pathology and Biochemistry, University of Colorado Medical School, Denver, Colo. (U.S.A.)

(Received June 13th, 1966)

SUMMARY

Suspensions of phospholipids were prepared from soya bean or mitochondria phosphatides or from purified egg lecithin. Using photometric, volumetric or gravimetric determinations it could be shown that the intraparticulate water present in these suspensions is lost by the addition of an osmotically active compound. The loss of intraparticulate water follows the Boyle–Van 't Hoff law. By extrapolation of these data it can be shown that all the intraparticulate water present in the suspension would be lost at infinite osmotic concentration. Very little intraparticulate water is lost when glycerol is used as the solute. Freezing and thawing of the suspension decreases the amount of water in the phospholipid particles. With 0.17 mM Ca^{2+} there is an increase in the amount of intraparticulate water lost in the presence of different concentrations of sucrose. At this concentration of Ca^{2+} no intraparticulate water is lost in the absence of sucrose indicating that the Ca^{2+} effect is not osmotic. 1.7 mM K^+ does not produce the effect observed with Ca^{2+}. Suspensions of phospholipids extracted from mitochondria swollen with ascorbate do not lose water in the presence of sucrose.

INTRODUCTION

Loss of water from mitochondria which have been swollen *in vitro* can occur either by an osmotic effect[1,2] or by an "active" mechanism requiring ATP[3,4]. A third type of water extrusion, which requires the addition of an equimolar mixture of divalent cations and chelating agents (hereafter called Me^{2+}–EDTA), has been demonstrated in dialyzed rat-liver mitochondria[5] and in other subcellular fractions[6]. The third type of reaction can also be elicited from swollen phospholipid suspensions in the presence of serum albumin[7]. These suspensions, when examined in the electron microscope, show irregular forms which can be described as vesicular and multilamellar[8]. The presence of considerable amounts of water can be detected by gravimetric determinations[7].

It is of importance to show whether the intraparticulate water can be lost

osmotically, and if the mechanism of action of Me^{2+}–EDTA is osmotic. Transport studies through lipid barriers were carried out with various systems: by forming a bimolecular leaflet[9–12], by impregnating collodion membranes[13,14] or Millipore filters[15–18] with lipids, or by studying phospholipid suspensions[19,20].

Using lecithin "spheres" EDELBERG[19] observed volume changes after changing the osmolality of the medium. BANGHAM, STANDISH AND WATKINS[20] predicted from their studies on the movement of ions and water using lecithin suspensions that an osmotic effect should be observable. In EDELBERG's experiments, NaCl was used as the osmotic solute. Since this compound precipitates phospholipid micelles[21,22], it seemed desirable to repeat the experiment using non-electrolytes as the osmotic solutes. Also, a more quantitative study of the volume changes due to the osmolality of the medium seemed desirable. It will be shown by three different techniques that phospholipid suspensions respond to the osmotic gradient and follow the Boyle–Van 't Hoff law. This system is in many aspects dissimilar to the reaction elicited by Me^{2+}–EDTA, and in this latter system no effect of the medium osmolality on the rate of the reaction is found.

Two similarities between the osmotic behavior of the phospholipid suspensions and natural systems are observed. It is known that natural membranes recognize the size of the solute molecule[23,24]; this property is also possessed by the phospholipid barrier. The presence of high Ca^{2+} concentrations in the medium decreases permeability of certain compounds and increases water loss from the cell[25–28]. In the presence of low Ca^{2+} concentrations that do not affect the water content of the phospholipid suspension, it is possible to demonstrate an increase in water loss in the presence of an osmotically active compound such as sucrose. A preliminary account of some aspects of this problem has been reported[29].

EXPERIMENTAL

Rat-liver mitochondria were prepared according to LEHNINGER[4]. Phospholipid suspensions were prepared as previously described without serum albumin[7]. Egg lecithin was prepared as described by PANGBORN[30] and further purified by column chromatography on silicic acid[31]. The purity of the material was checked by thin-layer chromatography[7]. Total lipids were extracted from mitochondria according to FOLCH-PI and dried under N_2.

A given amount of the suspension is placed in a cuvette and a given amount of the solute to be tested is added. The timing is started at this moment, the cuvette is mixed and the absorbance of the suspension is determined at 10 sec. The control cuvette contains the solute mixed with water. Protein was determined by the biuret reaction in the presence of deoxycholate[32]. Radioactivity was measured with a thin-end-window counter. Volumetric determinations of phospholipid suspensions were carried out in calibrated plastic tubes which were used in a swinging bucket rotor of the Servall SS-4 centrifuge. Gravimetric determinations of water content were obtained by weighing before and after drying of the centrifuged material. Interparticulate water was determined by adding a known amount of [^{14}C]inulin and determining the radioactivity of the pellet.

Soya been phosphatides were obtained from Associated Concentrates, Woodside, N.Y. [^{14}C]Inulin was obtained from Volk Radiochemical, Skokie, Ill. Dextran (aver-

age mol. wt. 250 000) was obtained from Sigma Chemical Co., St. Louis, Mo. Vasopression was obtained from Mann Research Laboratories, New York, N.Y.

RESULTS

Photometric determinations

Soya bean phosphatide suspensions show the same osmotic behavior towards sucrose as subcellular fractions when photometric techniques are used. The refractive index of the medium was kept constant with high-molecular-weight dextran. All the data shown have been obtained in at least four experiments. The data are expressed as the inverse of absorbance in agreement with the results reported by TEDESCHI and co-workers[1,2,33,34].

As shown in Fig. 1, at two different concentrations of sucrose, the inverse of absorbance is proportional to the inverse of the phospholipid concentration, calculated as μmoles of lipid-P. Because this relationship exists, it is possible to correct the values of $1/A$ of different experiments carried out with different concentrations of phospholipid. In the subsequent experiments the value of 1/lipid-P concentration was set to 1, *i.e.*, 1 μmole of lipid-P per incubation mixture. Similar data are obtained if the phospholipid suspension is dialyzed either for 2 days or with five hourly changes of dialysis fluid. Fig. 2 shows that at a given sucrose osmolality, the inverse of absorbance increases when the refractive index of the medium is raised.

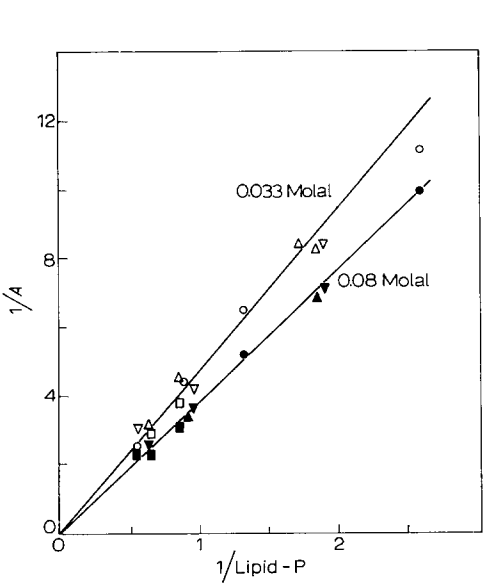


Fig. 1. Relationship between absorbance and phospholipid suspension concentration at two different sucrose concentrations. Abscissa: inverse of concentration of suspension; ordinate: inverse of absorbance 10 sec after mixing of sucrose and phospholipid suspension. Incubation at room temperature.

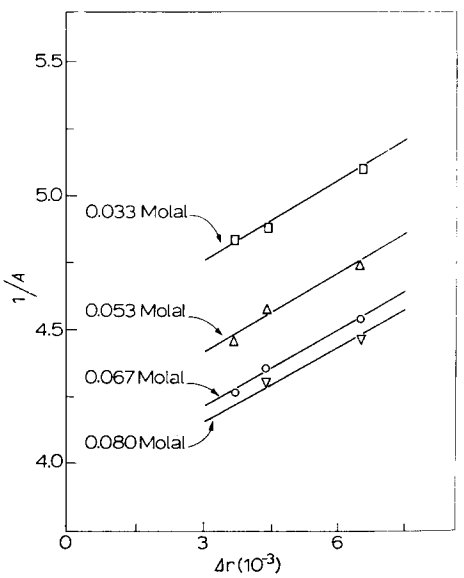


Fig. 2. Dependence on refractive index of medium of absorbance in relation to sucrose concentration. Abscissa: refractive index of medium; ordinate: inverse of absorbance. Determined as in Fig. 1. and data calculated for 1/lipid-P = 1.

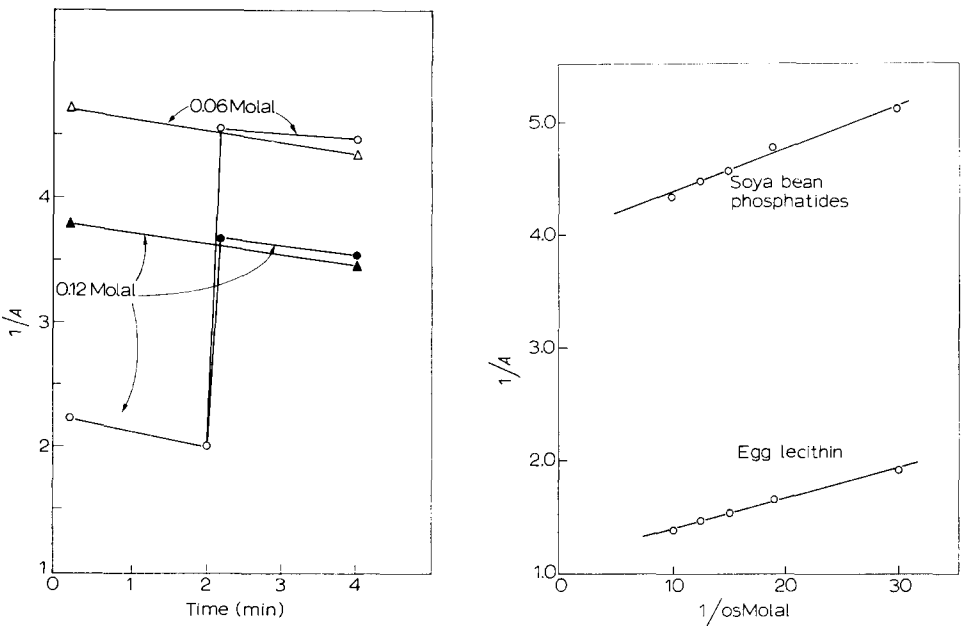


Fig. 3. Reversal of osmotic changes. Abscissa: time; ordinate: inverse of absorbance. Phospholipid concentration for first 2 min of ○——○ tracing is 2 μmoles, in all other cases is 1 μmole. For explanations see text.

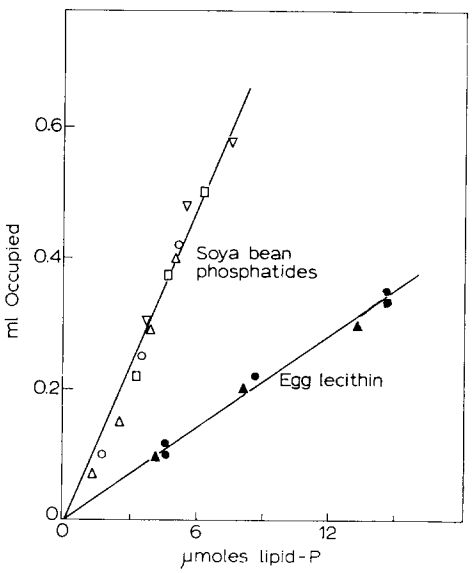
Fig. 4. Absorbance changes of suspensions of egg lecithin and of soya bean phosphatides in the presence of various sucrose concentrations. Abscissa: inverse of sucrose concentration; ordinate: inverse of absorbance. Determined as in Fig. 1 and calculated as in Fig. 2.

The reversibility of these absorbance changes is shown in Fig. 3. In Fig. 3 the phospholipid suspension is kept in 0.12 Molal sucrose, and at 2 min an equal volume of either 0.12 Molal sucrose or of solvent is added. The decrease of absorbance in the presence of 0.12 Molal sucrose represents only the dilution of the phospholipid while the larger decrease seen with solvent alone, represents both the dilution of the phospholipid and the increase of intraparticulate water in the phospholipid suspension due to the decrease of the sucrose osmolality. The values obtained after dilution are comparable to those of the phospholipid suspension at one-half the concentration mixed with the appropriate sucrose concentrations.

The data were repeated using different types of phospholipid preparations. Fig. 4 compares data obtained with suspensions prepared from soya bean phosphatides and from purified egg lecithin. It is evident that both preparations respond to sucrose according to the Boyle–Van 't Hoff law. The absolute values of the absorbances are consistently higher for the egg lecithin at any one sucrose concentration, suggesting that the latter preparation may contain less water. Total mitochondrial lipids also responded to sucrose concentration as the other phospholipid preparations. No great reproducibility is obtained with these preparations in the apparent intraparticulate water content of the dispersed suspension from one preparation to another although the suspensions were prepared in exactly the same way.

Volumetric determinations

This technique was used to check the photometric data shown above. In Fig. 5 it can be seen that the volume occupied by phospholipid suspensions is proportional to the amount of phospholipid present in suspension. The data shown in Fig. 6 indicate that the relationship between volume occupied and the inverse of osmolality holds true for soya bean phosphatide suspensions. It might be argued that the smaller volume occupied at higher sucrose concentrations is due to incomplete sedimentation of the phospholipid suspensions because of the higher density of the medium. This does not seem to hold true since at longer centrifugations of 90 min there is only a small increase of volume and it is evident at all concentrations of sucrose.



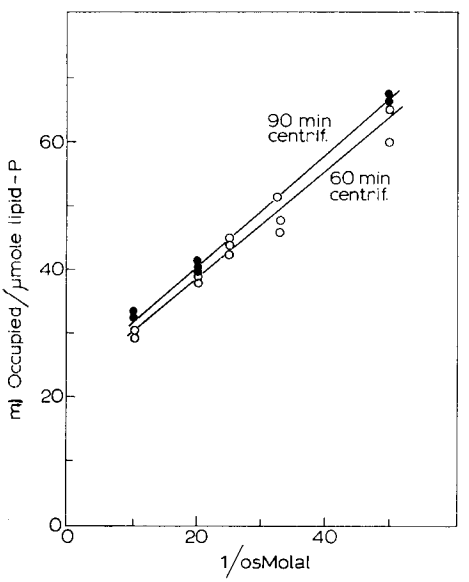


Fig. 5. Volume occupied by various concentrations of suspensions of egg lecithin or soya bean phosphatides. Abscissa; phospholipid concentrations; ordinate: volume occupied by the centrifuged pellet.

Fig. 6. Volume changes of soya bean phospholipid suspensions in the presence of various amounts of sucrose. Abscissa: inverse of sucrose concentrations; ordinate: volume occupied.

Gravimetric determinations

The amount of intraparticulate water is rather constant from one preparation to the other (see Table I). This amount decreases with longer centrifugation at higher speed. It also varies with the type of phospholipid used to prepare the suspension. Since similar evidence is obtained with photometric (Fig. 4) and volumetric (Fig. 5) techniques, these various data are compared. For the various data the ratio of the values of soya bean phospholipid to that of egg lecithin is around 3.

Evidence of the quantitative relationship between photometric data and amount of intraparticulate water present in the swollen phospholipid was obtained by determining the effect of freezing the suspension before use. This was carried out in an acetone–dry ice mixture; after thawing the suspension behaved osmotically towards sucrose. Freezing and thawing removes water (Table II), and the increase of

TABLE I

GRAVIMETRIC DETERMINATIONS OF WATER CONTENT OF VARIOUS PHOSPHOLIPID SUSPENSIONS

		mg water per μmole lipid-P	*% inulin space*	*Centrifugation*
Egg lecithin	1	12.6	14.2	1 h at 105 000 × *g*
	2	14.2	17.1	
	3	14.3	16.4	
Soy bean phosphatides	1	42.3	25.7	1 h at 105 000 × *g*
	2	45.5	19.4	
	3	49.4	23.2	
	1	32.3	26.9	80 min at 195 000 × *g*
	2	22.1	23.0	
	3	25.1	21.0	
	4	25.7	20.4	
	5	26.1	17.6	

absorbance correlates well with the loss of intraparticulate water. The ratio of these values lies between 6.5 and 7.5 (Table II). In Table II is seen that more intraparticulate water is lost by subsequent freezing. At present, no evidence for a minimum amount of water not affected by freezing has been obtained. The loss of intraparticulate water is prevented if freezing is carried out in 0.25 M sucrose but not in 0.125 M NaCl. More intraparticulate water is lost by quick freezing than by slow freezing. The frozen and thawed suspensions do not re-swell by dialysis overnight.

TABLE II

PHOTOMETRIC AND GRAVIMETRIC DETERMINATION OF WATER LOSS AFTER FREEZING OF PHOSPHOLIPID SUSPENSION

	Times frozen	*mg water* (a)*	*1/A* (b)*	*a/b*
1. Soya bean phosphatides	0	43.8	6.22	7.20
	1	19.3	2.57	7.54
	3	11.1	1.67	6.63
2. Soya bean phosphatides	0	35.8	5.01	7.15
	1	25.6	3.85	6.65
	3	13.8	1.86	7.42
3. Soya bean phosphatides	1	26.0	3.98	6.53
	3	8.1	1.24	6.52
4. Egg lecithin	0	13.7	1.97	6.95
	1	5.1	0.76	6.70
5. Egg lecithin	1	7.8	1.16	6.72

* Calculated per μmole lipid-P.

As mentioned above the photometric determinations of intraparticulate water loss using suspensions prepared from mitochondrial lipid are not quantitatively reproducible (see below also). Table III compares gravimetric and photometric determinations carried out with these preparations. Although the water content is unexplainably different, the ratio of the photometric data and the water content is quite

TABLE III

COMPARISON OF GRAVIMETRIC AND PHOTOMETRIC DATA OBTAINED WITH SUSPENSIONS OF TOTAL LIPID FROM RAT-LIVER MITOCHONDRIA

	mg water (a)*	*1/A at 0 Molal sucrose* (b)*	*1/A at 0.05 Molal sucrose* (c)*	*a/b*	*a/c*	*% inulin space*
I	14.5	1.58	1.32	9.2	10.9	25.0
II	18.4	1.98	1.61	9.3	11.4	25.4
III	21.4	2.07	1.78	10.3	12.0	23.3
IV	36.6	3.85	2.54	9.5	14.4	15.7

* Calculated per μmole of lipid-P.

similar for the various preparations. This again supports the evidence for the quantitative significance of the photometric determinations in this system.

It is also important to establish how much of the intraparticulate water present in these phospholipid suspensions is free and responds to the osmotic pressure. Fig. 7 indicates that all the water is affected by sucrose in the presence of $CaCl_2$ (see below for the effect of this compound) and that no evidence for bound water exists.

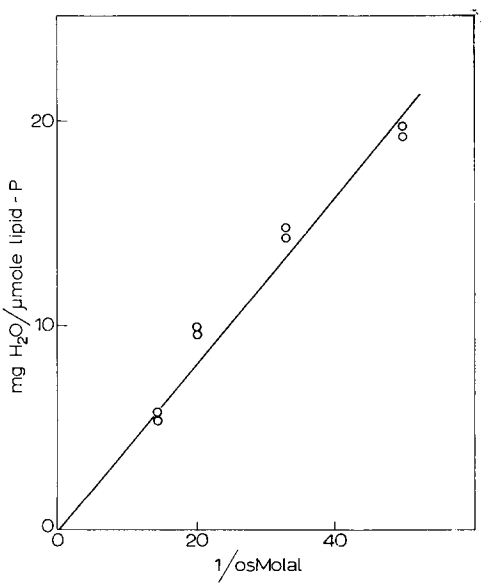


Fig. 7. Gravimetric changes of water content of phospholipid suspensions in the presence of various sucrose concentrations and 0.17 mM $CaCl_2$. Abscissa: inverse of sucrose concentration; ordinate: amount of intraparticulate water.

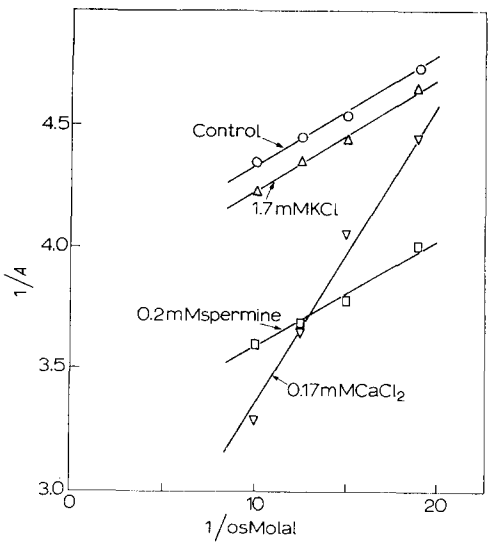


Fig. 8. Effect of various compounds on absorbance changes of phospholipid suspension in the presence of various sucrose concentrations. Abscissa: inverse of sucrose concentrations; ordinate: inverse of absorbance. Determined as in Fig. 1 and calculated as 1/lipid-P = 1.

Effect of various compounds on osmotic properties

A series of compounds were tested using the photometric assay in the presence of sucrose. Some of these are without apparent effect; *e.g.*, 0.1 M butanol, 0.01% deoxycholate, 0.04% Tween 60, 1 μM oleic acid, 33 μg pitressin (containing 2 pressor units) or 330 μg bovine serum albumin per incubation. The effect of some compounds which reproducibly affected the response to sucrose of the phospholipid suspensions are shown in Fig. 8. It is apparent that the various compounds do not alter the osmotic response, since that data follow the Boyle–Van 't Hoff law. It can be seen that in the presence of 0.17 mM Ca^{2+} there is a considerable increase in amount of intra-

TABLE IV

WATER CONTENT OF PHOSPHOLIPID SUSPENSIONS IN THE PRESENCE OF $CaCl_2$, $MgCl_2$, KCl, AND SPERMINE

	mg water/μmole lipid-P	
	No sucrose	*With 0.08 Molal sucrose*
1. Control	24.4	11.8
2. With 0.17 mM $CaCl_2$	26.2	6.9
3. With 1.7 mM KCl	21.7	9.1
1. Control	25.3	9.6
2. With 0.2 mM spermine	16.4	5.4
1. Control	25.9	9.8
2. With 0.17 mM $MgCl_2$	24.1	5.0

particulate water lost in the presence of sucrose. Effects similar to these shown by Ca^{2+} were obtained with Mg^{2+} and polylysine (3.3 μg/ml). The effect of Ca^{2+} is lost when it is added together with equimolar amounts of EDTA. The presence of 1.7 mM KCl does not alter the effect of the divalent cations. The effect of Ca^{2+} is not osmotic since no effect was seen in the absence of sucrose. 1.7 mM KCl or 0.2 mM spermine (see Fig. 8) increase absorbance in the absence of sucrose but do not affect the absorbance increases due to sucrose additions.

Gravimetric data on water content in the presence of various compounds are shown in Table IV indicating close agreement with the photometric determinations. The effect of KCl may possibly be of osmotic nature. It is possible that spermine effects the phospholipids in a way similar to the Me^{2+}–EDTA reaction previously described[5,7]. Since at this concentration Ca^{2+} does not affect the amount of water present in the suspension one should assume that it reacts mostly with the phosphate groups of the phospholipids of the outer layers. It has been suggested by PARPART[35] that the effect of Ca^{2+} on diffusion in natural membranes occurs by decreasing the

TABLE V

INTERPARTICULATE WATER OF PHOSPHOLIPID SUSPENSIONS IN THE PRESENCE OF VARIOUS COMPOUNDS

Additions	*mg water/μmole lipid-P*	
	Expt. I	*Expt. II*
1. Control	6.6	7.9
2. With 0.17 mM $CaCl_2$	4.7	2.7
3. With 1.7 mM KCl	7.2	7.9
1. Control	5.8	6.5
2. With 0.17 mM $MgCl_2$	5.4	6.3
3. With 3.3 μg polylysine per ml	5.6	6.6
1. Control	6.7	4.8
2. With 0.2 mM spermine	4.6	2.2

water present in the membrane. Evidence supporting this view is shown in Table V, where values for the interparticulate water are reported. The amount of interparticulate water is significantly decreased by the addition of Ca^{2+} and not by KCl, but, on the other hand, the effect of Mg^{2+} and of polylysine is too small to be significant.

Effect of solutes of different size

Since natural membranes are able to recognize the size of various solutes such as carbohydrates of various chain length, the effect of these compounds on the phospholipid suspensions was studied. For this purpose the photometric changes were followed with the use of a recording instrument and the effect of solutions of constant molality was tested. Fig. 9 shows tracings obtained after the exposure of the suspension to mannitol, erythritol and glycerol. The tracings start below 100% transmittance, since the solutions are added with the shutter open. In the presence of mannitol there is a decrease in transmittance indicating loss of intraparticulate water which then reaches

a steady level. The vertical lines in the tracings indicate moments at which the carrier is moved from the test sample to the control sample prepared in dextran of the same refractive index and back, and the lower tracings indicate values for the control which in this and the other tracings does not change. When erythritol is used as the solute a similar increase in absorbance is observed but instead of reaching a plateau there is a later decrease of absorbance. This suggests that this sugar is able to permeate the

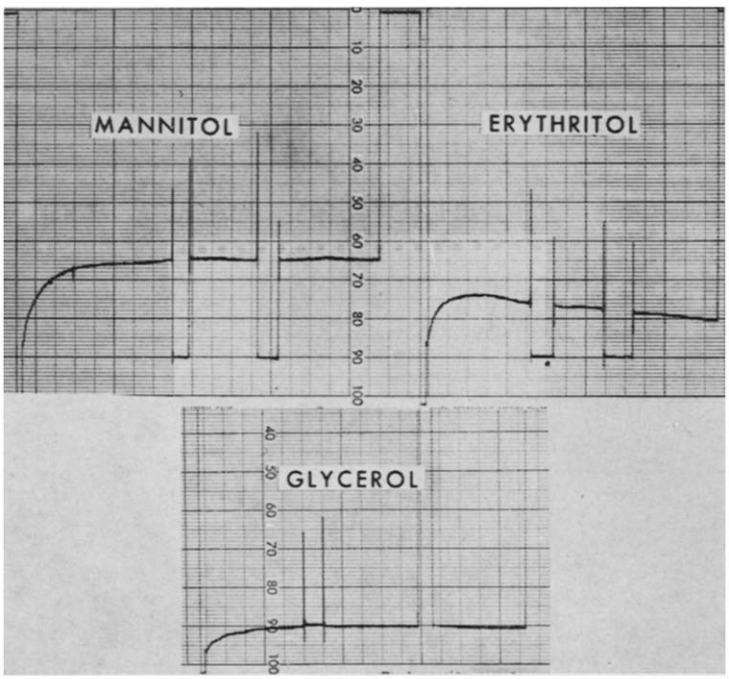


Fig. 9. Time course of absorbance changes of phospholipid suspensions in the presence of 0.08 Molal mannitol, erythritol or glycerol. Abscissa: time (15 sec between vertical lines); ordinate: per cent transmittance. For explanations, see text.

phospholipid suspensions, but that it goes at a slower rate than water; thus, water moves first out and only later on will it move back in with the solute. When glycerol is the solute added, then little if any increase of absorbance above the control value is seen. This suggests that glycerol penetrates the lipid barrier very fast.

Lack of osmotic response of lipids of ascorbate-swollen mitochondria

It has been shown by HUNTER *et al.*[36,37] that ascorbate produces swelling of rat-liver mitochondria with concomitant formation of peroxidation products of the lipids. Although the interrelationship between the two events is not clear, it can be argued that because of the peroxidation of unsaturated fatty acids of the phospholipid, the mitochondrial membrane becomes leaky. Fig. 10 shows that osmotic response to sucrose additions of ascorbate-swollen rat-liver mitochondria is present during the swelling process but that no effect is seen at the end of the swelling period. Total

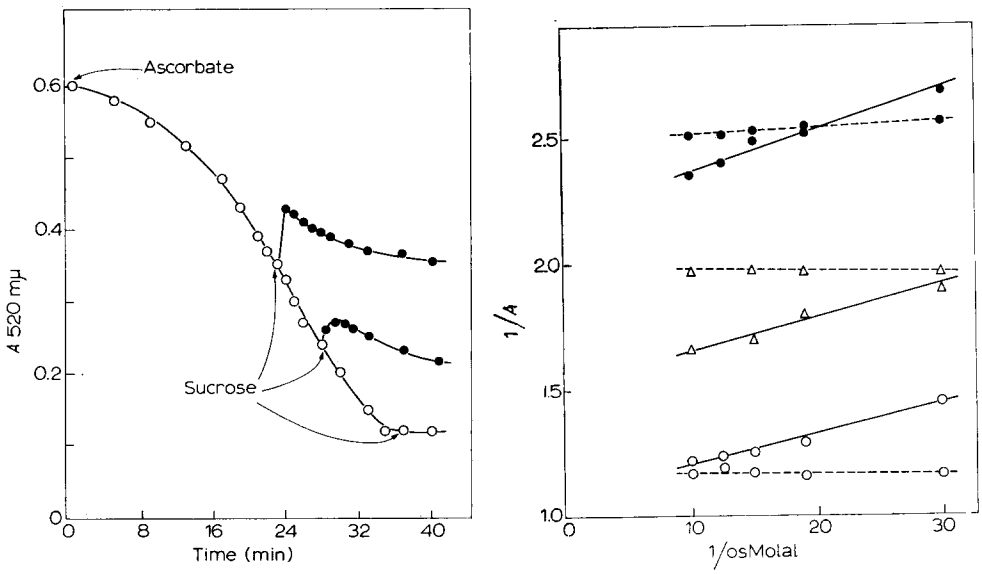


Fig. 10. Effect of sucrose additions at different time intervals after the addition of ascorbate to rat-liver mitochondria. Abscissa: time; ordinate: absorbance. Incubation mixture: 20 mM Tris–HCl (pH 7.4), 125 mM KCl, 0.3 mM EDTA in a final volume of 3 ml containing 2 mg serum albumin. Incubation at room temperature. At arrows enough sucrose to bring the solution to 70 mM was added in small volume.

Fig. 11. Absorbance changes of phospholipid suspension prepared from normal and ascorbate-swollen mitochondria in the presence of various sucrose concentrations. Abscissa: inverse of sucrose concentration; ordinate: inverse of absorbance. ●—●, △—△, ○—○, three different preparations of normal mitochondrial lipid; ●— —●, △— —△, ○— —○, three different preparations of ascorbate-swollen mitochondrial lipid. Determined as in Fig. 1 and calculated for 1/lipid-P = 1.

lipids were prepared from such mitochondria or from mitochondria incubated without ascorbate. Suspensions were prepared from each of these incubations and the response to sucrose was determined photometrically. Although the absolute values are not reproducible (Fig. 11), it is evident that all three preparations prepared from the ascorbate-swollen mitochondria are unaffected by the changes of sucrose osmolality.

DISCUSSION

The data presented here confirm the observation reported by EDELBERG[19] and indicate that loss of intraparticulate water from a phospholipid suspension in the presence of sucrose obeys the Boyle–Van 't Hoff law. Comparison of gravimetric, volumetric and photometric data indicate that in this system the photometric data are quantitatively significant. Thus, the experiments which indicate osmotic reversal (Fig. 3) and the effect of various sugars (Fig. 9) are valid. The lipid barriers of these preparations are semi-permeable in agreement with the results of BANGHAM, STANDISH AND WATKINS[20], and they recognize the size of the solute, *e.g.* from erythritol to glycerol. That a similar recognition pattern exists in red blood cells is apparent from the relative hemolysis time of 60 for glycerol and 10 750 for erythritol[23].

The effect of Ca^{2+} on the osmotic behavior of these suspensions is of particular

interest. Interaction between this ion and phospholipid has been observed through the study of phospholipid monolayers[38], micellar suspensions[21,22,39] and with Millipore filters impregnated with lipids[15–18]. With the last system, decreased osmotic water flux is observed in the presence of Ca^{2+} (see ref. 16). In the same system Ca^{2+} is observed to decrease the water content of the membrane model. Similar observations are made with the model system used here. Ca^{2+} increases the amount of water lost in the presence of sucrose and the extent of water loss still obeys the Boyle–Van 't Hoff law. Ca^{2+} decreases the amount of interparticulate water but not that present inside the particles. A diagramatic representation of the possible mechanism of Ca^{2+} is shown in Fig. 12.

The particles composing the suspension are represented as empty circles, but should be considered as multivesicular and multilamellar. The hydrophylic portion of the phospholipid of the outer layer of the particles are represented as P. On the right-

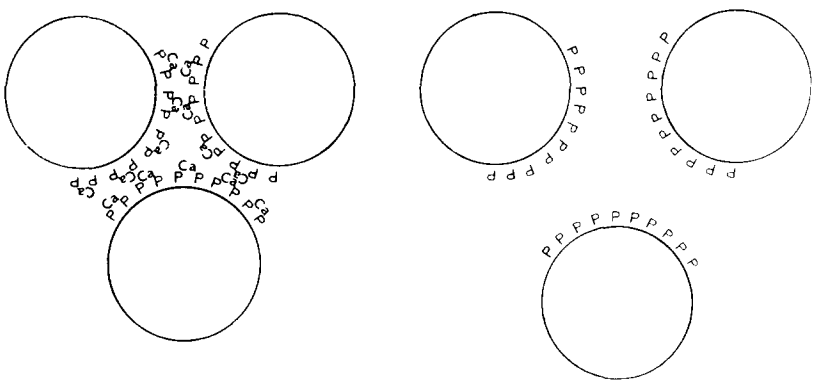


Fig. 12.

hand side, the particles are shown in the absence of Ca^{2+}; on the left side, in the presence of the cation. In the latter case, the distance between particles has decreased while the size of the particle has remained constant. No special emphasis should be given to the actual number of Ca^{2+} on the outer surface of the particle; it is only suggested that Ca^{2+} reacts with the outer portion of the phospholipid. The quantity of water lost under these conditions amounts to 105–288 molecules of water per molecule of phospholipid. Whether this water is actually present at all times bound to the surface of the particles is not known.

Freezing of the suspensions damages these structures with concomitant loss of water from the inside. This is of interest since the damaging effect of freezing on biological systems is well-known. The fact that more damage is observed with quick freezing than by slow freezing suggests the formation of intraparticulate ice crystals[40,41]. Various compounds such as glycerol and sucrose protect cells from the injurious effects of freezing. These compounds protect enzymes from inactivation by freezing[42] and lactate dehydrogenases from hybridization[43]. The effect of freezing in cells has often been considered in terms of the effects of freezing on protein or nucleic acid macromolecules. The observation presented here indicates that the lipid component of the cell is also affected by freezing, and that this effect can be prevented by sucrose.Since

lipids are present in membranes, this effect of freezing is of great importance in understanding the effect of freezing on cells like the erythrocyte, in which the membrane may be damaged[44,45].

The work of HUNTER *et al.*[36,37] indicates that certain agents such as Fe^{2+}, ascorbate and mixtures of GSH–GSSG swell mitochondria with formation of peroxidation products of lipids. These workers also show formation of lipid peroxidation by some of these agents when mixed together with pure fatty acid[46]. A product of lipid peroxidation is malonaldehyde but this compound does not produce swelling[37]. Thus the data suggest that peroxidation of lipid components *per se* will induce permeability changes of the mitochondrial membrane. Evidence in support of this hypothesis is shown by the photometric determinations of the response to sucrose by total lipid suspensions extracted from normal mitochondria and from ascorbate-swollen mitochondria (Fig. 11). The lipid preparation from the ascorbate-swollen mitochondria did not respond to sucrose addition indicating that this sugar probably enters the suspension at a considerably higher rate. This seems to be the first instance in which it is possible to test some of the permeability properties of components of natural membranes which have been damaged *in situ*.

The osmotic properties of these suspensions and the Me^{2+}–EDTA-elicited water extrusion system are compared. It has been previously reported[5,7] that Me^{2+}–EDTA-dependent water extrusion does not behave osmotically. The data reported here support the previous contention since no effect of increased osmolality on the rate of water extrusion is observed. Also, osmotic loss of water is not affected by serum albumin and EDTA which are required for water extrusion by the lipid suspensions[7]. Ca^{2+} has an effect on both water extrusion and osmotic loss of water, but various data suggest a difference in the mechanism. 0.17 mM Ca^{2+} has no effect on the intraparticulate water content of lipid suspensions, but at this concentration it enhances the amount of intraparticulate water lost at any given sucrose concentration. The effect by Ca^{2+} on osmotic loss of water is not altered by the presence of serum albumin, however it is abolished by equimolar concentrations of EDTA. Both serum albumin and EDTA are required for water extrusion elicited by Ca^{2+} from these preparations[7].

Water extrusion and osmotic loss of water from these suspensions are due to different mechanisms. Although Ca^{2+} probably interacts with the phosphate groups of the phospholipid, the mechanism by which it affects these two reactions appears to be different.

ACKNOWLEDGEMENTS

The skillful technical assistance of Miss U. MAURER is appreciated. This work was aided by grants from the U.S. Public Health Service AM09963 and CA05981.

REFERENCES

1 H. TEDESCHI, *Biochim. Biophys. Acta*, 46 (1961) 159.
2 H. TEDESCHI AND H. J. HEGARTY, *Biochem. Biophys. Res. Commun.*, 19 (1965) 558.
3 A. L. LEHNINGER, *J. Biol. Chem.*, 234 (1959) 2187.
4 A. L. LEHNINGER, *J. Biol. Chem.*, 234 (1959) 2465.
5 R. RENDI AND N. MCCAIG, *Arch. Biochem. Biophys.*, 104 (1964) 267.
6 N. MCCAIG AND R. RENDI, *Biochim. Biophys. Acta*, 79 (1964) 416.
7 R. RENDI, *Biochim. Biophys. Acta*, 84 (1964) 694.

8 A. E. VATTER, *J. Cell Biol.*, 19 (1963) 72A.
9 P. MUELLER, D. O. RUDIN, H. TI TIEN AND W. C. WESCOTT, *Nature*, 194 (1962) 979.
10 P. MUELLER AND D. O. RUDIN, *J. Theoret. Biol.*, 4 (1963) 268.
11 C. HUANG, L. WHEELDON AND T. E. THOMPSON, *J. Mol. Biol.*, 8 (1964) 148.
12 T. HANAI, D. A. HAYDON AND J. TAYLOR, *J. Gen. Physiol.*, 48 Suppl. (1965) 59.
13 J. H. WEATHERBY, *J. Cellular Comp. Physiol.*, 21 (1943) 1.
14 J. H. WEATHERBY, *J. Cellular Comp. Physiol.*, 33 (1949) 333.
15 J. M. TOBIAS, D. P. AGIN AND R. PAWLOWSKI, *J. Gen. Physiol.*, 45 (1962) 989.
16 G. J. LEITCH AND J. M. TOBIAS, *J. Cellular Comp. Physiol.*, 63 (1964) 225.
17 D. C. MIKULECKY AND J. M. TOBIAS, *J. Cellular Comp. Physiol.*, 64 (1964) 151.
18 R. RENDI AND A. R. REID, *J. Cell Biol.*, 19 (1963) 58A.
19 R. EDELBERG, *J. Cellular Comp. Physiol.*, 41 (1953) 37.
20 A. D. BANGHAM, M. M. STANDISH AND J. C. WATKINS, *J. Mol. Biol.*, 13 (1965) 238.
21 D. C. ROBINS AND I. L. THOMAS, *J. Pharm. Pharmacol.*, 15 (1963) 157.
22 M. B. ABRAMSON, R. KATZMAN AND H. P. GREGOR, *J. Biol. Chem.*, 239 (1964) 70.
23 R. HÖBER AND S. L. ØRSKOV, *Arch. Ges. Physiol.*, 231 (1933) 599.
24 D. A. GOLSTEIN AND A. K. SOLOMON, *J. Gen. Physiol.*, 44 (1960) 1.
25 G. WHITTEMBURG, N. SUGINO AND A. K. SOLOMON, *Nature*, 197 (1960) 699.
26 P. F. CURRAN, F. C. HERRERA AND W. J. FLANIGAN, *J. Gen. Physiol.*, 46 (1963) 1011.
27 C. S. TIDBALL, *Am. J. Physiol.*, 206 (1964) 243.
28 W. E. LASSITER, A. FRICK, G. RUMRICH AND K. J. ULLRICH, *Arch. Ges. Physiol.*, 285 (1965) 90.
29 R. RENDI, *J. Cell Biol.*, 27 (1965) 83A.
30 M. C. PANGBORN, *J. Biol. Chem.*, 188 (1951) 471.
31 R. M. C. DAWSON, *Biochem. J.*, 88 (1963) 414.
32 E. E. JACOBS, M. JACOB, D. R. SANADI AND L. B. BRADLEY, *J. Biol. Chem.*, 223 (1956) 147.
33 H. TEDESCHI AND D. L. HARRIS, *Biochim. Biophys. Acta*, 28 (1958) 392.
34 H. TEDESCHI, J. M. JAMES AND W. ANTHONY, *J. Cell Biol.*, 18 (1963) 503.
35 A. K. PARPART, *Cold Spring Harbor Symp. Quant. Biol.*, 8 (1940) 25.
36 F. E. HUNTER, JR., J. F. LEVY, J. FINK, B. SCHUTZ, F. GUERRA AND A. HURWITZ, *J. Biol. Chem.*, 234 (1959) 2176.
37 F. E. HUNTER, JR., A. SCOTT, P. E. HOFFSTEN, F. GUERRA, J. WEINSTEIN, A. SCHNEIDER, B. SCHUTZ, J. FINK, L. FORD AND E. SMITH, *J. Biol. Chem.*, 239 (1964) 604.
38 D. O. SHAH AND J. H. SCHULMAN, *J. Lipid Res.*, 6 (1965) 341.
39 H. S. HENDRICKSON AND J. G. FULLINGTON, *Biochemistry*, 4 (1965) 1599.
40 H. T. MERYMAN. *Science*, 124 (1956) 515.
41 P. MAZUR, *Biophys. J.*, 3 (1963) 323.
42 K. SHIKAMA AND I. YAMAZAKI, *Nature*, 190 (1961) 83.
43 O. P. CHILSON, L. A. COSTELLO AND N. O. KAPLAN, *Federation Proc.*, 24, Suppl. 15 (1965) S-55.
44 P. MAZUR, *Federation Proc.*, 24, Suppl. 15 (1965) S-175.
45 G. RAPATZ, J. NATT AND B. LUYET, *Biodynamica*, 9 (1963) 83.
46 J. WEINSTEIN, A. SCHNEIDER AND F. E. HUNTER, JR., *Biochem. Biophys. Res. Commun.* 11 (1963) 452.