SWELLING OF FISH MITOCHONDRIA

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ABSTRACT

The physical properties of fish liver and rat liver mitochondria were compared as a function of temperature and osmotic pressure. The data indicate that fish mitochondria are more flexible and swell at a more rapid rate over a 0 to 30°C temperature range, whereas the rates of swelling at 30 to 40°C are comparable. The swelling rates of both fish and rat mitochondria vary with temperature and approximate the Arrhenius relationship. Apparent energies of activation for swelling averaged 26.5 kcal and 12.9 kcal for rat and fish, respectively. Fish mitochondria were less stable than rat mitochondria to osmotic variation, and the disparity in initial swelling rates became increasingly greater with lower osmotic pressure. The hypotonic swelling of both fish and rat mitochondria was readily reversed osmotically; however, there was a very rapid decay of reversal in fish mitochondria and only a very slow decay in the case of rat. All the data indicate that under comparable conditions the fish mitochondrial membranes are more flexible and presumably more permeable and labile than rat mitochondrial membranes. The findings are discussed in relation to the general metabolic implications and the possible contributions of the membrane constituents to membrane behavior.

INTRODUCTION

Apparently, mitochondria are bounded by a typical biological membrane which obeys osmotic law and is capable of swelling up to 5 osmotic volumes (1). Swelling or shrinking may be relevant to the control of cellular metabolism, since substrates oxidized by the respiratory chain augment mitochondrial swelling whereas compounds which inhibit electron transport counteract this effect (2–5). The physical behavior of mitochondrial membranes has thus attracted considerable attention because of this possible relationship with metabolic regulation. In view of this, a study of the osmotic properties of mitochondria from poikilothermic and homeothermic animals may reveal differences in physical behavior as reflected by their metabolic requirements. Possibly, poikilothermic animals such as fish, metabolizing at widely varying temperatures, would have different membrane requirements from those of homeothermic animals.

In addition, fatty acid analyses of fish and rat liver mitochondria indicate that fish mitochondria contain more highly unsaturated fatty acids and presumably fatty acids of the linolenate rather than linoleate series (6). Differences in the physical properties of the fatty acids within the phospholipids of the membrane might thus favor differences in the physical behavior of the mitochondrial membranes as required by environment and metabolism.

This paper reports the comparative physical behavior of fish liver and rat liver mitochondria as functions of osmotic pressure and temperature and considers the data in terms of the general metabolic implications and the physical properties of the fatty acids in the mitochondrial membranes.

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EXPERIMENTAL

Mitochondria were isolated by differential centrifugation in 0.44 M sucrose according to the procedure outlined by Dounce *et al.* (7). The pH of the homogenates was maintained between 6.0 and 6.5 by the addition of 0.1 M citric acid. The procedure was modified to exclude the initial debris-washing step; however, sufficient 0.44 M sucrose was added to maintain the same approximate homogenate concentration. De-ionized water and reagent grade chemicals were used throughout the experiments. In each experiment, washed liver mitochondria from 3 adult Long-Evans rats, 10 to 20 white catfish (*Cattus albus*), or 7 to 10 bluegill (*Lepomis macrochirus*) fish were suspended in sufficient 0.44 M sucrose so that 0.1 to 0.2 ml in 5 ml gave an absorbancy reading of about 0.7. The swelling experiments were performed according to the procedure of Lehninger *et al.* (8). Sucrose solutions were prepared in 0.02 M Tris buffer, pH 7.4. The swelling was measured by the decrease in absorbancy at 520 mμ in the Bausch and Lomb Spectronic 20. Tubes were matched with a standard bentonite suspension to ± 1 per cent T. Temperatures were maintained constant by incubating the mitochondria in large Dewar flasks of water. Readings were then taken at the indicated intervals. Measurements at 0° and 10°C were less reliable than those at higher temperatures because of the tendency for moisture to condense on the tubes in the cell compartment. The condensation problem was overcome by rapidly transferring the suspensions

TABLE I

Cytochromes in Rat and Fish Liver Mitochondria

Mitochondria	Cytochromes (moles/mg protein × 10^{10})			
	a	*b*	*c*	*a3*
Rat	0.9	0.9	1.7	1.8
Catfish*	1.9	1.1	1.7	2.9
Bluegill*	1.2	0.9	1.2	1.6

* From the data of Richardson *et al.* (12).

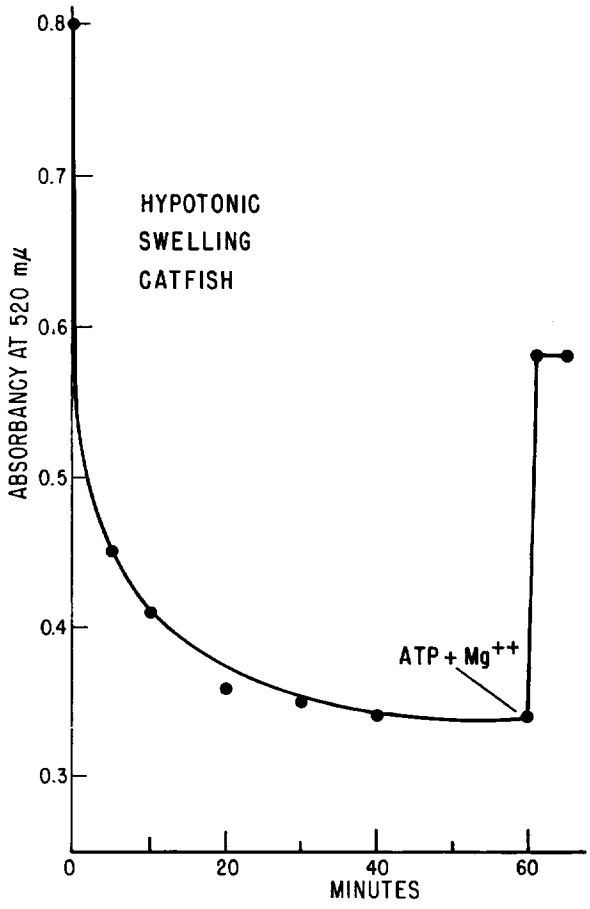

FIGURE 1

Reversal of hypotonically swollen catfish liver mitochondria. Test system contained 5.0 ml of 0.02 M Tris buffer, pH 7.4; mitochondria; and addition of ATP, 0.005 M, plus $MgCl_2$, 0.003 M.

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to a tube positioned for reading and then quickly returning the suspension to the water bath. This led to some slight fluctuation in the incubation temperature and probably accounts for some of the variations in swelling rates observed at low temperatures. Ordinarily, it required less than 30 seconds to take a reading.

Osmotic reversal experiments were performed essentially as described by Tedeschi and Harris (9). Briefly, this involved hypotonic swelling in 2.5 ml 0.02 M Tris buffer, pH 7.4, followed by addition of 2.5 ml 0.8 M sucrose in Tris buffer at timed intervals. The rate of the decay of swelling was then followed at 520 mμ.

Mitochondrial protein was determined by the method of Gornall *et al.* (10).

Cytochrome contents of the mitochondria were calculated from difference spectra after dithionite reduction using the extinction coefficients and wavelength pairs of Chance and Williams (11).

Mitochondrial fatty acids were isolated and their methyl esters were analyzed by gas-liquid chromatography using the Aerograph A-90-C as described before (12).

RESULTS

Difference spectra of liver mitochondria from rat, catfish, and bluegill indicate a qualitative and quantitative similarity, as shown in Table I. The cytochrome contents are similar to those reported by Estabrook and Holowinsky (13) for rat and guinea pig liver mitochondria. These data, considered with the mode of fractionation, indicate that the mitochondria are reasonably pure.

Isolation of catfish liver mitochondria in 0.25 M sucrose yielded particles that gave erratic swelling results. Possibly the mitochondria were preswollen in this medium, since isolation in 0.44 M sucrose gave more reproducible results.

Fig. 1 illustrates the rapid swelling of catfish

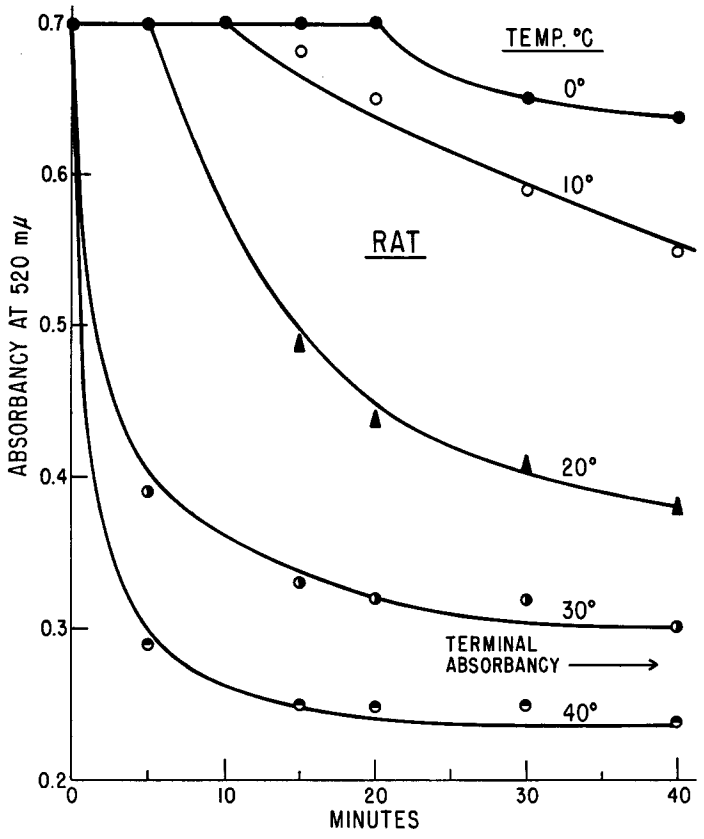

FIGURE 2

Kinetics of spontaneous swelling of rat liver mitochondria. Standard test system of 5.0 ml 0.3 M sucrose —0.02 M Tris, pH 7.4, plus mitochondria at indicated temperatures.

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liver mitochondria in a hypotonic medium and the instantaneous reversal by ATP and magnesium ions. The response is quite analogous to that observed for rat liver mitochondria (14).

Fig. 2 shows the kinetics of rat liver mitochondrial swelling as a function of temperature. These data are very similar to those reported by Lehninger *et al.* (8). At the lower temperatures there is a considerable lag in swelling, up to 20 minutes at 0°C. The lag disappears at 30°C, and the overall rate of swelling increases very rapidly. The relatively slow rate of swelling at lower temperatures would imply that the membranes are physically rigid, presumably reflecting the physical properties of the membrane components. By comparison, the swelling rates of mitochondria from catfish in Fig. 3 are not so greatly affected by temperature. Readily evident is the lack of a lag period at the lower temperatures. These data illustrate a basic difference in the physical behavior of mitochondrial membranes of catfish as compared with rat. Bluegill liver mitochondria behaved very similarly to catfish liver mitochondria, exhibiting no lag period at low temperatures and swelling at comparable rates as a function of temperature. From these data it is evident that at higher temperatures in the 30 to 40°C range the swelling rates of mitochondria from fish are comparable to those of rats; but in the lower ranges from 0 to 30°C the fish mitochondria swell at a much faster rate.

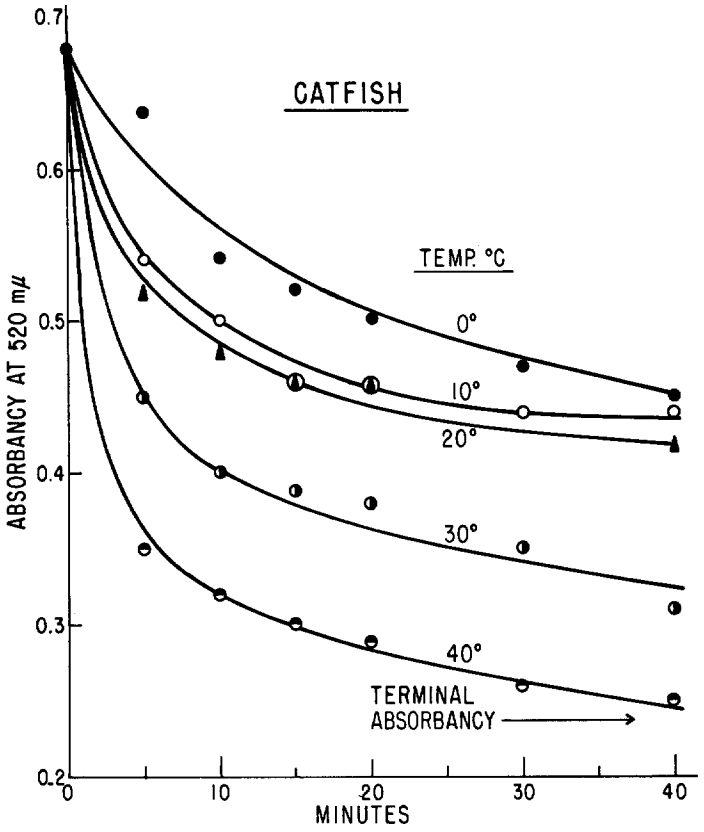

FIGURE 3

Kinetics of spontaneous swelling of catfish liver mitochondria. Standard test system of 5.0 ml 0.3 M sucrose—0.02 M Tris, pH 7.4, plus mitochondria at indicated temperatures.

Application of the kinetic treatment used by Lehninger *et al.* (8) allows a more quantitative examination of the swelling process. Assuming

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that maximal swelling is approximated by the maximal extent of swelling at 40°C, initial rates of swelling can be derived from the time required to reach one-fourth maximal swelling, and the reciprocal of this rate leads to an initial rate constant for swelling. Analysis of the rate constants indicates that the swelling rate of fish mitochondria between 0 and 30°C is about tenfold that of rat mitochondria. However, in the 30 to 40°C range the rates are comparable. A plot of the logarithm of the rate constant and the reciprocal of the absolute temperature shown in Fig. 4 indicates that the swelling follows approximately the Arrhenius relationship. The plots represent two separate experiments on rat liver mitochondria and four separate experiments on catfish liver mitochondria. The agreement between experiments, particularly with the catfish mitochondria, is reasonably good considering the complexity of the system. Although there are evident variations in swelling rates between temperature increments, a composite curve can be drawn to illustrate the swelling behavior of catfish liver mitochondria. The slopes of the plots for the swelling of rat liver mitochondria agree quite well with a similar plot of the data of Lehninger *et al.* (8). Apparent energies of activation for swelling as calculated from the Arrhenius equation yield the range of values in Table II. These values serve to put the differences in the physical behavior of the mitochondria on a more quantitative basis. It is evident from these data that the temperature effects of swelling are considerably less in fish than in rats. This would be expected on the basis of probable metabolic needs of the fish. In addition, the swelling rates of rat and fish are comparable in the 30 to 40°C range, while the swelling rates of fish are 10 times higher in the lower

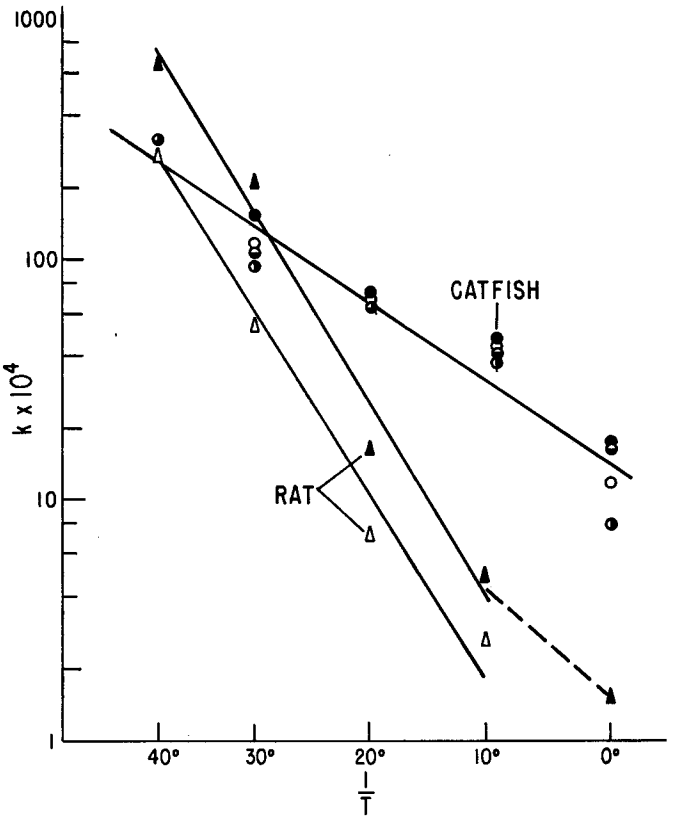

FIGURE 4

Arrhenius plots for kinetics of swelling of catfish and rat liver mitochondria in standard test system.

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temperature ranges. The fish membranes are apparently more permeable to polar water and sucrose molecules at a given temperature.

A second indicator of relative membrane behavior is the reaction of the mitochondrial membranes to changes in osmotic pressure at constant temperature. As shown in Fig. 5, the rat mitochondria swell sluggishly in 0.4 M sucrose, and as the osmotic pressure of the system is decreased, the swelling rate increases. By comparison (Fig. 6), the rate of swelling of fish mitochondria is more affected by variation of osmotic pressure. The rate of swelling is greater than that of rat liver mitochondria at 0.4 M sucrose and increases more as the osmotic pressure is decreased. This is illustrated in Fig. 7, where the initial rate constants for swelling of fish mitochondria are greater than those for rat mitochondria, especially at low sucrose concentrations. In this case, the initial rate constants for swelling were derived as before, assuming hypotonic swelling as maximal. In both cases the rate of swelling in 0.1 M sucrose was too fast to allow an approximation of the initial swelling rate. Also, the swelling of fish and rat mitochondria in 0.8 M sucrose was similar in that there was an instantaneous drop in the initial absorbancy followed by a very slow apparent swelling. It is readily apparent that the fish mitochondria respond more rapidly to variation in osmotic pressure of the medium at a given temperature. These data would indicate that fish membranes are more flexible and permeable than those from rat liver.

A third test of the relative physical behavior of

TABLE II

Apparent Energies of Activation for Mitochondrial Swelling

Liver mitochondria	Temperature (C)	Energy of activation (kcal/"mole")
Rat*	10–40°	23.6
Rat	10–40°	27.3
Rat	10–40°	28.8
Catfish	0–30°	11.4
Catfish	0–30°	12.2
Catfish	0–40°	12.5
Catfish	0–40°	15.7
Bluegill	0–40°	7.0

* Calculated from the data of Lehninger *et al.* (8).

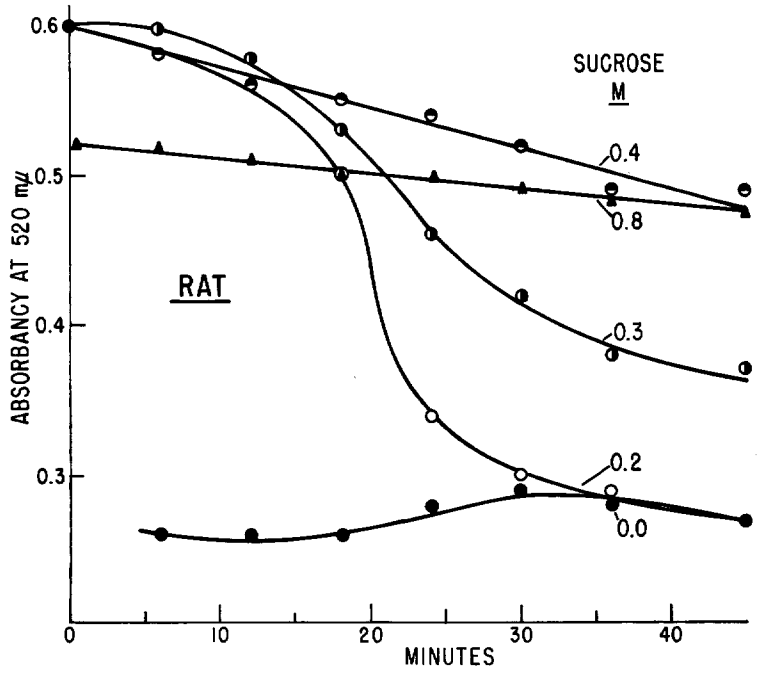

FIGURE 5

Effect of sucrose concentration on spontaneous swelling of rat liver mitochondria. Medium was 0.02 M Tris, pH 7.4, and indicated sucrose concentrations; temperature, 20°C.

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fish mitochondria is the osmotic reversal of swelling. The mitochondria were swollen in a hypotonic medium for varying lengths of time and the medium was adjusted to 0.4 M sucrose by addition of an equal volume of 0.8 M sucrose. The reversal of swelling and its subsequent decay at 20°C are illustrated in Figs. 8 and 9 for rat liver and catfish liver mitochondria, respectively. Both mitochondria show an osmotic reversal of approximately the same magnitude. In the case of the rat, the decay is very slow after reversal. On the other hand, the decay is extremely rapid in fish, returning to the maximal swelling in about 6 minutes. These data on osmotic reversal again indicate the greater flexibility and osmotic lability of fish mitochondrial membranes.

Analyses of mitochondrial fatty acids from catfish and rat livers are listed in Table III. A different fatty acid pattern is evident upon comparing the data. The catfish mitochondria contain the longer chain and more highly unsaturated fatty acids characteristic of fish and apparently of the linolenate series (6, 15). On the other hand, the fatty acids of rat liver mitochondria are less unsaturated and are primarily linoleate and arachidonate.

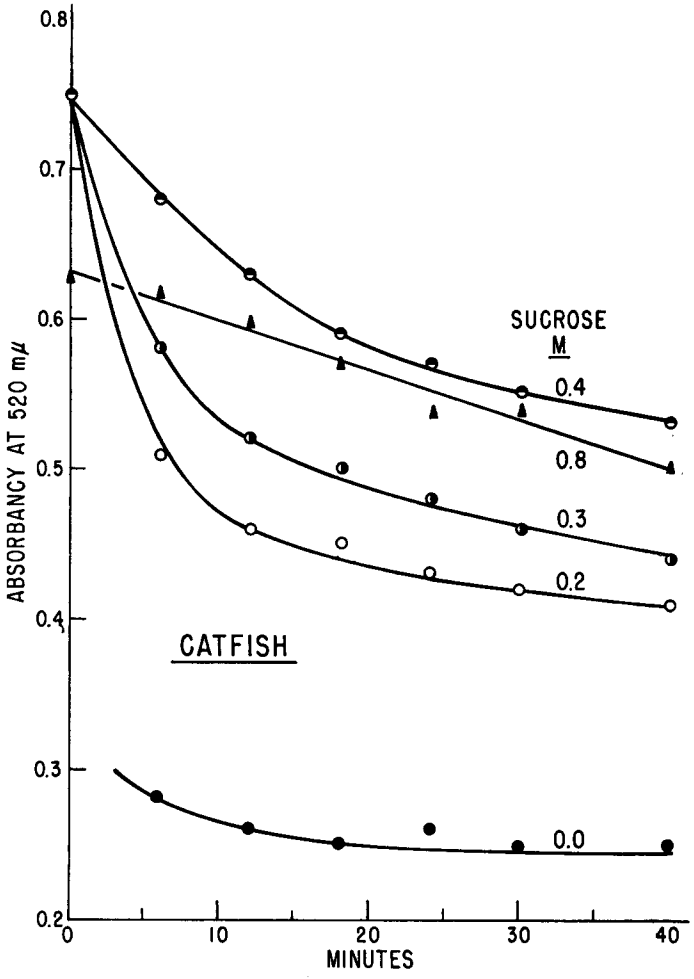

FIGURE 6

Effect of sucrose concentration on spontaneous swelling of catfish liver mitochondria. Medium was 0.02 M Tris, pH 7.4, and indicated sucrose concentrations; temperature, 20°C.

DISCUSSION

The experiments described here appear to be quite suitable for comparing swelling properties of mitochondrial membranes. One reason is the vast amount of comparative literature on mitochondrial swelling in sucrose solutions. In virtually every instance there are pronounced differences in the physical properties of mitochondrial membranes as between catfish liver and rat liver.

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The reasons and requirements for these differences between fish and rat mitochondria are no doubt very complex for such complicated systems. The metabolic implications of this difference may be profound; however, it is difficult to draw any definitive conclusions. The data might reflect the metabolic needs of each animal as dictated by its environment.

given temperature indicates that fish mitochondria are more susceptible to osmotic shock and are more permeable to polar water and sucrose molecules. The same holds true for the relative rates of swelling following osmotic reversal.

Consideration of the present data at a more fundamental enzymatic level is not possible; however, it may be that the difference in apparent

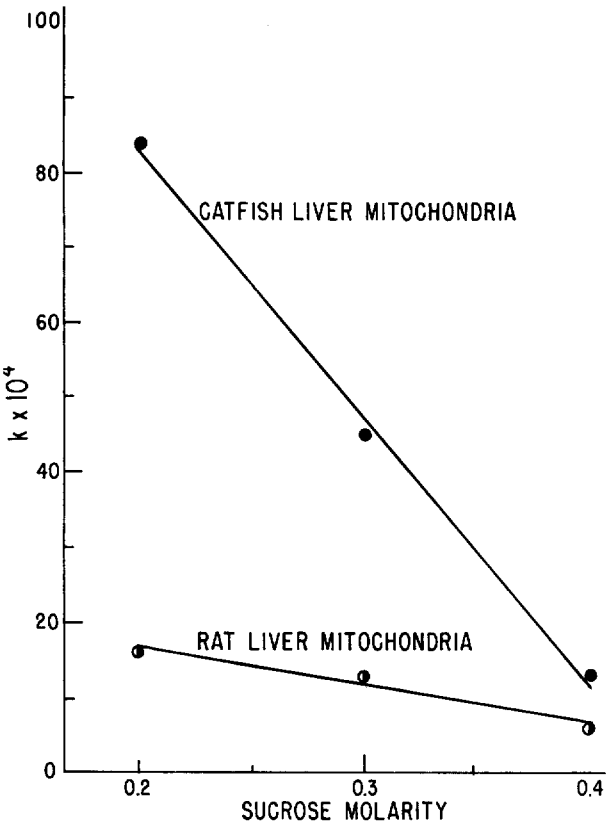

FIGURE 7

Variation of spontaneous swelling rates of ra and catfish liver mitochondria as a function o osmotic pressure.

It is evident that the physical responses of fish mitochondria are consistent with possible requirements of a fish as imposed by its environment and metabolism. Presumably, fish must adjust their metabolism to a range of temperatures and generally must function at temperatures lower than 37°C. If swelling and contraction of mitochondria are involved in metabolic regulation, it would be to the advantage of the fish to have membranes more flexible at lower temperatures and less subject to temperature effect.

The relative behavior of fish and rat mitochondria toward variation in osmotic pressure at a

energy of activation for swelling may reflect differences in enzyme systems involved in the swelling process. For example, a fish phospholipase with a lower apparent energy of activation might release the U factor proposed by Wojtczak and Lehninger (16) more rapidly at lower temperatures, yielding a more rapid rate of swelling. On the other hand, the response to variation in osmotic pressure might mean that sucrose is less inhibitory (8) to fish enzymes involved in the swelling process.

In the passive systems used in these studies and in the absence of exogenous substrates it might be

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profitable to examine the responses in the light of the constituents of the mitochondrial membranes. The greater flexibility of the membrane at the lower temperature would presumably reflect the physical properties of the materials in the mitochondrial membrane. The concept of a biological membrane in general (17) and the mitochondrial membrane in particular (1) as a bimolecular phospholipid leaflet between two layers of protein within the interior of the membrane. In this case the non-polar fatty acid residues would interact and the flexibility and physical properties of the membranes would then in part be governed by their physical interactions within the interior of the membranes. From the analyses of Holman and Widmer (19) there is no apparent compartmentalization of particular fatty acids within submitochondrial particles. Consequently, we can

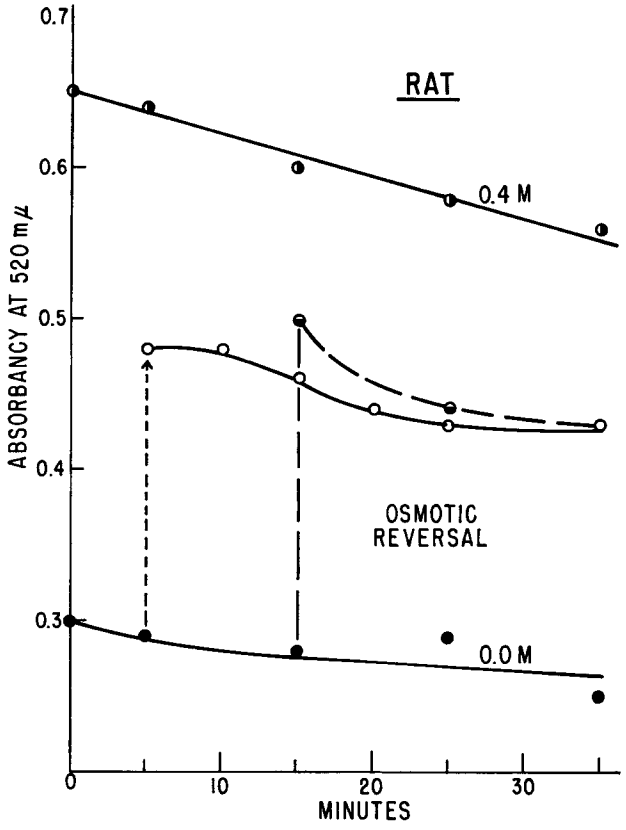

FIGURE 8

Osmotic reversal of hypotonic swelling of rat liver mitochondria. Test system contained 2.5 ml 0.02 M Tris buffer, pH 7.4; mitochondria; and added 2.5 ml 0.8 M sucrose in 0.02 M Tris buffer, pH 7.4, at indicated times.

allows some consideration of the differences in physical behavior of the membranes. Membrane stability and flexibility would then depend to a large extent on the physical properties of the proteins and phospholipids in the membrane. Although data are not available for fish mitochondrial proteins, fish proteins are less stable than the corresponding mammalian proteins (18) and may contribute to the relative membrane instability. Presumably, the phospholipids would be oriented to allow electrostatic interaction with the proteins and mutual van der Waals interactions consider fatty acid analyses of whole mitochondria as reflecting the composition within the membrane.

Previous analyses of the mitochondrial fatty acids in rat and fish indicate that the fatty acids from fish are more unsaturated (6). These analyses were confirmed in the fatty acid analysis of the mitochondria used in these studies as listed in Table III. These analyses indicate a greater unsaturation of the fatty acids in catfish liver mitochondria, amounting to about a 15 per cent increase based on the double-bond index calculated as before (6). Presumably, the unsaturated

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fatty acids within the fish membranes would be of the linolenate rather than linoleate series (15), with the exception of the linoleate and arachidonate. From the analyses in Table III, there is an apparent ratio of linoleate to linolenate families of 10:1 and 0.26:1 in rat liver and fish liver mitochondria, respectively. Differences in unsaturation and fatty acid types are even more striking in previous analyses (6, 12). Consideration of the effects of unsaturation and double-bond positions on the physical properties of the fatty acids (20, 21), such as melting point, suggests that the types of fatty acids in the mitochondrial membrane would contribute to the flexibility and permeability of the membrane. The greater unsaturation of fish fatty acids, in addition to the terminal double bond's being only 3 carbons removed from the methyl tail, would favor a less rigid and more flexible membrane. On the assumption that the entry of highly polar water and sucrose molecules into the mitochondria is dependent on the polarization of double bonds, the more unsaturated linolenate type fatty acid would favor a more permeable membrane.

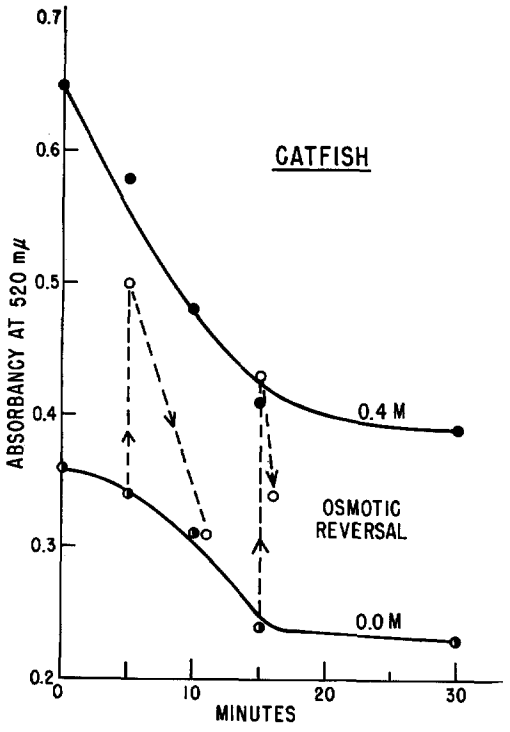

FIGURE 9

Osmotic reversal of hypotonic swelling of catfish liver mitochondria. Test system contained 2.5 ml 0.02 M Tris buffer, pH 7.4; mitochondria; and added 2.5 ml 0.8 M sucrose in 0.02 M Tris buffer, pH 7.4, at indicated times.

TABLE III

Mole Per Cent Mitochondrial Fatty Acids

Fatty acids*	Average retention volume	Rat liver	Catfish liver
14:0	0.337	0.3	0.5
14:1	0.399	0.0	0.1
15:0	0.435	0.2	0.0
15:1(?)		0.0	0.1
16:0 Br	0.502	Trace	0.2
16:0	0.571	16.0	14.1
16:1	0.656	1.8	4.4
17:0 Br	0.650	0.0	3.2
17:0	0.743	0.5	1.5
17:1	0.860	0.2	0.4
18:0 Br	0.850	0.0	0.8
18:0	1.00	18.2	11.2
19:0 Br		0.6	0.0
18:1	1.12	10.8	24.2
18:2	1.32	22.4	0.7
19:0	1.27	0.0	0.5
19:1	1.46	0.0	1.0
18:3, 20:0	1.70	0.4	1.2
18:4, 20:1	1.92	0.5	2.2
20:2	2.23	0.0	0.9
20:3	2.53	0.7	0.0
20:4	2.85	23.4	6.9
20:5	3.61	0.0	13.5
22:5	6.14	0.0	1.2
22:6	6.97	4.0	11.3

* Carbon chain:number of double bonds

The variation in physical behavior of mitochondrial membranes as a function of unsaturation and type of fatty acids in membrane phospholipids conceivably might have some significance relative to cold acclimation. For example, as blowfly larvae (22) become acclimated to lower temperatures the degree of unsaturation of their phospholipids increases. More specifically, there is an apparent difference in the mitochondria of goldfish acclimated (23) to different temperatures. Mitochondria from fish acclimated at 10°C appear to be uncoupled at 20°C, while those acclimated at 30°C are not. Kanungo and Prosser (23) attribute this to a basic alteration of the enzyme systems dur-

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ing the acclimation process. Another possible interpretation is that low temperature mitochondria are more unsaturated and thus more permeable at higher temperatures. In this case uncoupling could be a result of simple leakage of the membrane and subsequent hydrolysis of an activated phosphate.

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