J. Biosci., Vol. 2, Number 2 June 1980, pp. 87–98. © Printed in India.

Adaptation to salinity by fish: Alterations in energy transducing status of muscle mitochondria

INDU BASHYAM, SURESH NARAYAN and J. JAYARAMAN
Department of Biochemistry, School of Biological Sciences,
Madurai Kamaraj University, Madurai 625 021

MS received 3 November 1979; revised 8 March 1980

Abstract. The functional status of fish muscle mitochondria during exposure of the organism to salinity stress was studied. No alterations were observed in the substrate oxidation capacity. However, the mitochondria appear to be in an uncoupled state during the first few days of exposure and recover thereafter. This could be correlated to high endogenous Ca^{2+} levels in the mitochondria in the early period. A Na^{+} – Ca^{2+} antiport in these mitochondria is shown. Evidence is also presented to show that the mitochondria isolated during the early stages of stress are in a swollen state and are unable to contract on addition of ATP and Mg^{2+} Continued exposure to the stress, however, reverses the situation.

Keywords. Fish; salinity adaptations; muscle mitochondria; respiratory status; calcium uptake swelling and contraction.

Introduction

The classicial picture of mitochondria as the ATP synthesizing unit is fast being replaced by one envisaging it as a multi-functional unit, which transduces the chemical energy of substrate oxidation to different functions, like ATP synthesis, ion uptake, conformational changes, proton pumps, active transport etc. Thus there is *prima facie* evidence for channelized transduction and the question arises as to whether this channelization is regulated by the physiological and environmental exigencies of the cell.

A survey of mitochondria isolated from muscle tissues of some arthropods (Honnappa *et al*., 1975) and from the mantle tissues of molluscs (Hannah Sulochana *et al*., 1977a) had shown a possible correlation between the Ca^{2+} uptake capacity and the calcium need of the organism. Carafoli and Lehninger (1971) have also compared the interaction of Ca^{2+} with mitochondria from different tissues and species in an attempt to understand the relevance of calcium uptake by mitochondria. Another comparative survey in this laboratory (Honnappa, unpublished results) of the mitochondrial properties from different organisms, gave preliminary evidence for channelization of energy transduced and this varied with the physiology of the organism. We have also reported earlier (Hannah Sulochana *et al*., 1977b) that mitochondrial functions respond to environmental stress conditions imposed on

Publication No. 034 from the Department of Biochemistry.

the organism. In this study, the fresh water fish *Tilapia mossambica* (now renamed *Sarotherodon mossambicus*) was exposed to iono-osmotic stress, by transferring them from fresh water to 25% sea water. At various stages of transfer, we have isolated the mitochondria from the muscle tissue and studied the energy transducing functions of the organelle. Since the stress imposed was iono-osmotic in nature, we have concentrated our attention on two major energy transducing functions of the organelle, namely calcium uptake swelling and contraction.

Materials and methods

The conditions for collection of fish, acclimation to laboratory conditions (fresh water media) and direct exposure to stress conditions were as described earlier (Hannah Sulochana *et al.,* 1977b).

Isolation of mitochondria

The procedure used was the same as that reported earlier (Hannah Sulochana *et al.*, 1977b). The final mitochondrial pellet was washed once and suspended in 0.25 M sucrose. For swelling and contraction studies, 0.05 to 0.1 ml of the suspension (approximately 1 mg protein/ml) in 1 ml medium with an absorbancy of 0.4 at 520 nm were used.

Analytical methods

Protein was measured by Lowry's method using bovine serum albumin as the standard (Lowry *et al.*, 1951). In the case of sucrose density gradient analysis, Bradford's method (Bradford, 1976) was used for obtaining the protein profile.

Oxygen uptake was measured polarographically using a Clark type electrode attached to an Elico (India) X-Y recorder. The reaction medium and other conditions were the same as that reported earlier (Honnappa *et al*., 1975). For the NaCl treatment, samples containing the same amount of mitochondrial protein used for the Polarographic assay were taken. NaCl (20 mM) was added and the mixture was incubated for 3 min, followed by centrifugation at 10000 *g* in a Janetzki TH 12 centrifuge for 5 min. The pellets were washed twice with buffer and then used for oxygen uptake studies.

Mg^{2+} -ATPase activity was estimated according to the method of Epstein and Whittam (1966). The reaction medium (1 ml) contained 2 mM $MgCl_2$, 2 mM ATP, 8 mM imidazole acetate (pH 7.4). Mitochondria were added to initiate the reaction. After 10 min, the reaction was stopped by the addition of 0.3 ml of ice cold 10% trichloroacetic acid. The samples were kept in ice for 10 min and then centrifuged for 2 min at 10000 g. Inorganic orthophosphate in the supernatant was estimated according to the method of Fiske and Subbarow (1925). For the density gradient analysis, a discontinuous sucrose gradient containing 20, 30, 40, 50, 60, 65, 75% sucrose (total volume 4.5 ml) was used. Mitochondria, 0.1 ml—0.2ml. (about 560 μg protein) was loaded. The centrifugation was carried out at 108000 *g* in a VAC 601 ultracentrifuge for 1 h. Fractions were collected and succinate dehydrogenase and protein estimations were made in each sample.

For the measurement of endogenous ion level, mitochondria were extracted with 0.5 N HCl by boiling in a water bath for 15 min. The extract was centrifuged at 10000 g for 5 min. and the ions K^+, Na^+ and $C2^+$ were estimated in the supernatant by flame photometry.

The swelling experiments were performed according to Richardson and Tappel (1962) with slight modifications. Sucrose solutions were prepared in deionised, double distilled water. Mitochondria were added to the required sucrose media and swelling was measured by the decrease in absorbance at 520 nm in a Beckman DK-2 ratio recorder. Temperature was maintained at a constant value. The rate of swelling was calculated from the slope of the curve. Contraction of the swollen mitochondria was produced by the addition of a mixture of 5 mM ATP and 5 mM $MgCl_2$ after swelling the mitochondria in the sucrose medium.

Results

Respiratory capacity

Fishes were transferred from fresh water (tap water with negligible ionic concentration) to 25% sea water as mentioned in materials and methods. After different days of exposure, samples of fishes were taken out, muscle tissue excised out and mitochondria were isolated. The results obtained with muscle mitochondria are given in table 1. As can be seen, up to 14 days of exposure, no significant variations were observed

Table 1. Respiratory status of muscle mitochondria during long term exposure to 25% sea water stress.

Mitochondria†	Treatment	natoms oxygen uptake/ units min/mg. mitochondrial protein		ADP/O	R.C.	Mg^{2+} dependent ATPase levels. (umol Pi/min/mg protein)
		Succinate	+ Dinitrophenol			
Fresh water		5.8	11.5	1.8	2.0	0.74
1 day		6.3	6.3	0	1.0	
2 days		6.4	6.4	0	1.0	0.33
7 days		5.7	5.7	0	1.0	0.33
9 days		4.2	5.4	0	1.0	0.46
14 days		6.8	11.4	1.7	2.0	0.50
24 days		6.4	11.5	1.8	2.0	0.70
Fresh water	Calcium loading	5.8	n.d.	0	1.6	n.d.
7 days	NaCl	5.7	8.4	1.6	2.0	n.d.
9 days	NaCl	6.3	8.4	1.5	2.0	n.d.

† – The period of exposure to 25% sea water
n.d –Not determined
R.C. – Respiratory control index

in the succinate oxidation rate (state II). However, a different picture was seen when the dinitrophenol stimulated respiration rate (state V), respiratory control index and ADP/O ratios were taken into account. Starting on the very first day of exposure, the parameters were not measurable upto about the ninth day. But on subsequent exposure, on the 14th day, stimulation by dinitrophenol, respiratory control index and ADP/O ratios were restored to normal values.

ATPase activity

The Mg^{2+} -dependent ATPase activity during the course of stress is also given in table 1. It is obvious, that there is a drastic reduction (two-fold) in the activity soon after exposure to stress. The activity remains low till about the ninth day and thereafter recovers back to the control (fresh water) level rapidly by about the 24th day. These results fit well with the changes observed in ADP/O ratio and respiratory control values as mentioned above. The enzyme activities in post-mitochondrial supernatant fractions were also measured throughout and they remained at a steady low value of 0.1 μ mol P_i/min/mg protein throughout the period.

Density gradient analysis

The various mitochondrial samples were also subjected to sucrose density gradient centrifugation for further characterisation. The density gradient profiles are shown in figure 1.

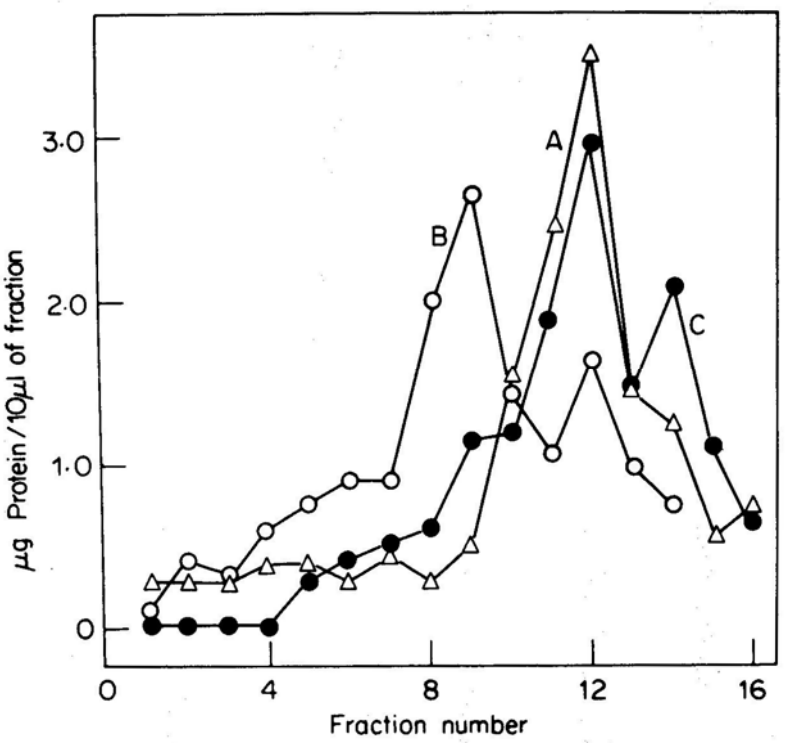

Figure1. Density-gradient analysis of Tilapia muscle mitochondria. (A) in fresh water. (B) On 7th day in 25% sea water. (C) Adapted to 25% sea water (exposed for more than 25 days).

Mitochondria from fresh water fish tissues showed essentially a single peak with succinate dehydrogenase activity at a density of 1.2175 g/cm^3. There was a small shoulder with no enzyme activity. Thus the preparations used in the studies were fairly pure. Mitochondria from muscle tissues of fishes exposed to 25% sea water for 7 days, however, showed two peaks at densities of 1.2296 g/cm^3 and 1.2175 g/cm^3, and both peaks had succinate dehydrogenase activity. When mitochondria were isolated 25 days after exposure the pattern was shifted. The heavier peak shifted to low density corresponding to that of fresh water samples and there was also a lighter peak (density 1.2170 g/cm3) containing succinate dehydrogenase activity.

One possible reason for the lighter density mitochondria becoming heavier, could be the loading of mitochondria with calcium (Lehninger, 1965). That this indeed is the case was shown by measuring of Ca^{2+} levels in the mitochondria (see below). A proportion of the mitochondria remained free of Ca^{2+} which is reflected by the second peak (density 1.2175 g/cm^3). It was interesting to find that on longer exposure to stress, the mitochondrial density returned to the normal value with the concomitant decrease in calcium levels (see also figure 2). The appearance of a peak at lower density 1.2170 g/cm^3 with succinate dehydrogenase activity could support the contention of new mitochondria being formed in this phase but this must await further experimentation.

Endogenous levels of ions

A study of the levels of different ions in these mitochondria was next carried out (figure 2). There were significant changes in the levels of Na^+ and Ca^{2+}, whereas

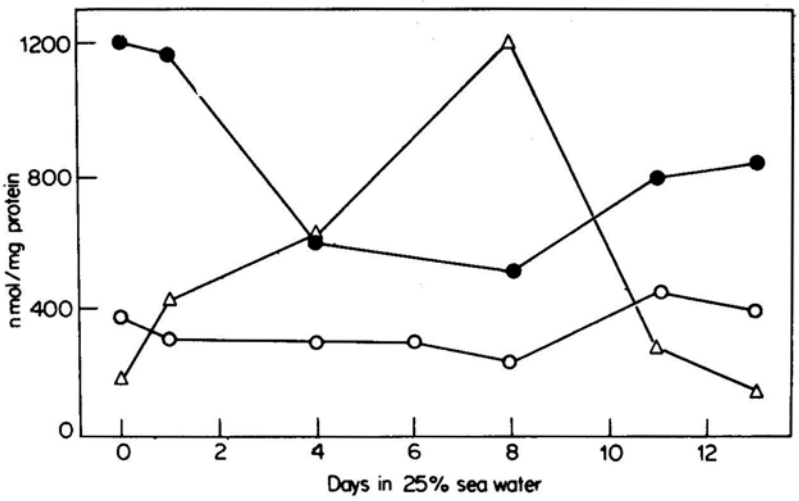

Figure 2. Endogenous levels of ions in mitochondria during continued exposure to 25% sea water. (Δ) Calcium. (●)Sodium. (O) Potassium.

K^+ levels showed only minor changes. The Na^+ levels expressed as nmol/mg mitochondrial protein decreased from 1200 to about 600 in 4 days, remains constant at this level till about 8 days and then slowly increased to 800 by about the 14th day. On the other hand, Ca^{2+} showed an inverse relationship to Na^+, rising sharply from the fresh water value of about 200 to 1200 on the 8th day, and returning to the low level on the 13th day. It should be mentioned at this point that only the total amount of the ions have been measured and no attempt was made to distinguish between soluble ionic state and precipitated salts, especially for calcium.

Na^+—Ca^{2+} interactions

It has been shown earlier (Crompton *et al.,* 1976; Carafoli *et al.,* 1974) that sodium inhibits the uptake of $^{45}Ca^{2+}$ by mitochondria and that incubation of calcium loaded mitochondria in a medium containing Na^+ , releases the calcium. We have been able to repeat these experiments with fish muscle mitochondria. The results of an experiment where the $^{45}CA^{2+}$ uptake by mitochondria isolated from fresh water fish in the presence of increasing concentrations of Na^+ in the medium showed a linear decrease as the Na^+ concentration in the medium increases from 0 to 2.25% (0 to 0.5 M) The $^{45}Ca^{2+}$ uptake decreases about 6-fold. In another series of experi ments, we have observed the total eflux of $^{45}Ca^{2+}$ from calcium loaded mitochondria, within 5 min of incubation in a Na^+ containing medium (results not given). Crompton *et al.* (1978) have shown a sigmoidal efflux of Ca^{2+} in the presence of Na^+ . The reasons for the differences between these results and the present studies need further investigation.

Based on these observations, the following series of experiments were designed. Fishes were exposed to 25% sea water as before and on the 8th day, the muscle mitochondria were isolated. The respiratory capacity, the respiratory control and ADP/O ratios were determined using the oxygen electrode. An aliquot of the mito-chondria was treated with NaCl as described in materials and methods, and the above mentioned parameters were measured. Using a third aliquot, the parameters were measured but with the addition of NaCl (20 mM) to the assay medium. NaCl treatment of the mitochondria (by either method mentioned) restored the ADP/O ratio, respiratory control and dinitrophenol stimulated respiration (table 1). No attempts were made in these experiments to ascertain if any stoichiometric relation ships were existent.

In another set of experiments, mitochondria from freshwater fishes were loaded maximally with Ca^{2+} (Hannah Sulochana *et al.*, 1977b) centrifuged, quickly washed and suspended in the poloragraphic assay medium. These mitochondria showed decreased ADP/O ratios and respiratory control. Further they responded to NaCl treatment as shown by the restoration of these activities (table 1).

Swelling patterns of mitochondria

The osmotic swelling and energy dependant contraction of mitochondria were next studied.

(i) Response of freshwater mitochondria to in vitro changes in osmolarity: It was found that in 0.25 M sucrose medium muscle mitochondria isolated from fresh water fishes gave fairly reproducible and steady swelling rate. Lower concentrations gave

varying results while at higher concentrations (upto 0.45 M sucrose) the rate was low. In all further experiments 0.25 M sucrose was used as the medium.

(ii)Passive swelling rate during exposure to salinity: Upon exposure to stress condition during the first 4 days, mitochondria showed a slight increase in the swelling rate (table 2). But remarkably between the 7th and 9th days, there was a

Table 2. Passive swelling rate of mitochondria.

Days after exposure to 25% sea water	Absorbance change (520 nm/min)
0	−0.1
2	−0.15
4	−0.15
7	+0.20
8	+0.15
9	+0.05
10	O
14	−0.20
21	−0.25

– value indicates swelling and
+ value indicates contraction.

contraction of the mitochondria rather than swelling. On longer exposure, the swelling properties regained their normal swelling response. This indicates a significant change in the internal osmotic environment of the muscle mitochondria during the initial phase of exposure to stress. Interestingly the rate of swelling of these mitochondria in 0.025 M sucrose media showed a very slight increase thereafter remaining constant (data not shown).

(iii) Contraction of muscle mitochondria

The addition of ATP—Mg^{2+} mixture to osmotically-swollen mitochondria instantaneously reverses the swelling and induces contraction. This response is analogous to that observed for rat liver mitochondria (Lehninger, 1959). Figure 3A shows a typical pattern of the absorbance changes during the initial swelling and the contraction following the addition of ATP-Mg^{2+}, by normal fresh water mitochondria. The extent of the reversal of swelling depends on the ratio of ATP/Mg^{2+} added. In the case of freshwater muscle mitochondria, it was seen that a ratio of >1 induced further swelling, whereas ratios of > 1 induced contraction. A ratio 1:1 of ATP and Mg^{2+} (5 mM each) gave optimal and reproducible results. Moreover, the contraction process was sensitive to bongkekric acid (95% inhibition at 10 μ M) and oligomycin (50-55%) in hibition at 10 μ M).

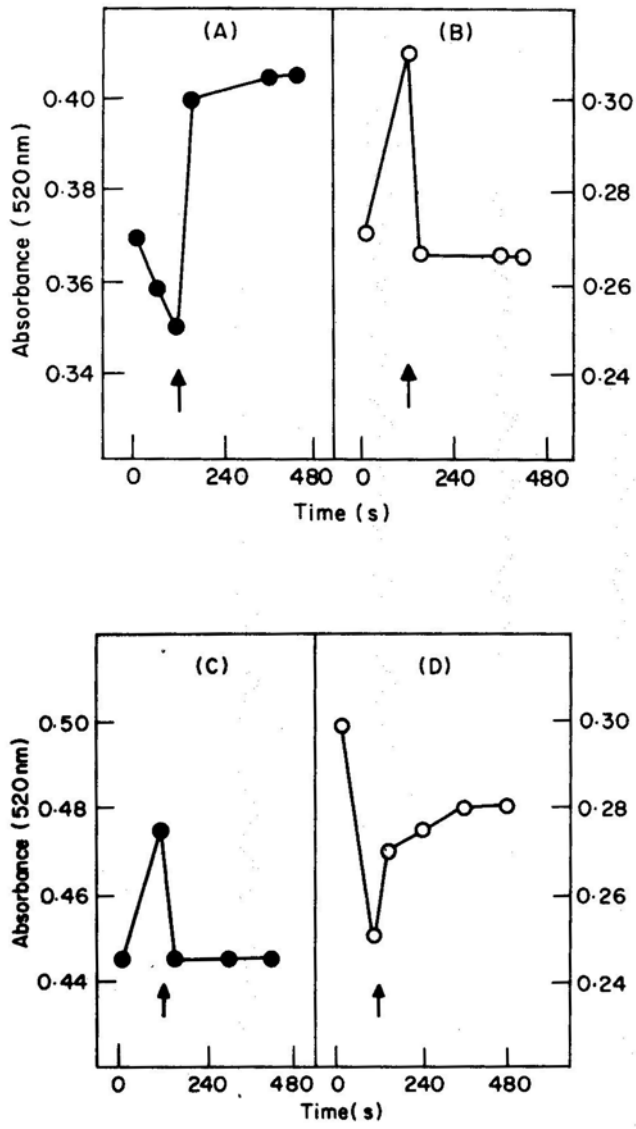

Figure 3. Patterns of mitochondrial swelling in 0.25 M sucrose and effect of addition of ATP Mg^{2+} mixture during continued exposure to 25% sea water.
5 mM ATP and 5 mM $MgCl_2$ were added at the time indicated (↑).
(A) Fresh water (B) 7th day in 25 % sea water. (C) 8th day in 25% sea water. (D) 20th day in 25% sea water.

The ATP induced swelling and its reversal by Mg^{2+} observed in these mitochondria, unlike rat liver mitochondria but similar to that reported by Dow *et al.* (1976)

for muscle mitochondria, was indeed interesting. Table 3 shows the rate of absorbance

Table 3. Mitochondrial contraction pattern during long term exposure to 25% sea water.

Days in sea water	Absorbance change at 520 nm on addition of ATP/Mg^{2+} mixture	Absorbance change at 520 nm on addition of ATP	Absorbance change at 520 nm on subsequent addition of Mg^{2+}
0 (fresh water control)	+0.05	0.086	0.09
7	−0.05	0.19	0.008
14	+0.03	0.12	0.07
20	+0.02	0.096	0.048
24	+0.03	0.08	0.086

ATP and Mg2+ were added at a concentration of 5 mM each.

changes on the addition of 5 mM ATP, followed by 5 mM Mg^{2+} to mitochondria after osmotic swelling in 0.25 M sucrose. The changes on the addition of the mixture of ATP/Mg^{2+} (5 mM each) to the mitochondria swollen in the same medium is also given. It is clear from the data presented that the ATP-induced swelling is completely reversed by Mg^{2+}. The addition of ATP/Mg^{2+} mixture brings about an instantaneous contraction.

(iv) Mitochondrial contraction pattern during long time exposure to salinity stress

Figure 3 shows the pattern of osmotic swelling in 0.25 M sucrose medium and the effect of addition of ATP/Mg^{2+} in mitochondria of fishes from the 7th, 8th and 20th days of exposure to 25% sea water stress. As mentioned earlier, the swelling pattern is significantly altered around the 8th day of stress and these preparations showed contraction in 0.25 M sucrose. It can be seen from the figure that the addition of ATP/Mg^{2+} to these contracted mitochondria induced an instant sharp decrease in absorbance, or swelling after which there was no further change in absorbance. The pattern of swelling and contraction of mitochondria from fishes exposed to 25% sea water stress for 20 days showed a reversal of this situation and the pattern resembled that of fresh water mitochondria.

The absorbance changes induced by ATP addition and the reversal of these by Mg^{2+} was also checked in mitochondria from the fishes under stress conditions. As is seen in table 3, the ATP induced swelling of these mitochondria increases until the eighth day, whereas there is a decrease in the ability of Mg^{2+} to reverse this function. The results indicated an increasing insensitivity of the mitochondria towards Mg^{2+} until 8-10 days of exposure to the stress.

The sensitivity of the process to oligomycin also reflected the same changes. However, the mitochondria was inhibited (90-95%) by bongkekric acid.

The results described above strongly indicated a change in the osmotic status of the mitochondria during initial stages of exposure to stress. The drastic reversal of the normal swelling pattern in 0.25 M sucrose observed in the mitochondria from samples on 7th to 9th day of exposure to stress indicated a swollen state of the mitochondria.

Discussion

On the various phases of mitochondrial response

On exposure of fresh water fish to a higher salinity (iono-osmotic stress) drastic deviations occurred in the functions of the mitochondria isolated from the muscle tissue leading to non-steady states. Two phases of this non-steady state could be clearly distinguished from the pattern of various mitochondrial functions. An initial response phase lasting for about 8 days (under the conditions of stress used), was characterized by changes in mitochondrial functions. These changes involved a loss in mitochondrial phosphorylation capacity, alterations in swelling and contraction, changes in endogenous levels of ions and *in vitro* Ca^{2+} uptake capacity as shown earlier (Hannah Sulochana *et al*., 1977b). This phase is a function of the stress. All these deviations could be elicited earlier by intensifying the stress.

A second (regulatory phase occurs during which mitochondria apparently regain their original status and all the parameters measured reverted back to normal levels. It seemed likely that during this second phase of adaptation, physiological mechanisms like hormonal interactions were probably involved and the cellular environment was also brought back to normal.

On the significance of calcium loading

In recent years, there has been considerable interest on the role of cellular calcium and its physiological relevance (Blum and Hoffmann, 1972; Carafoli and Crompton, 1978). Mitochondria have been envisaged as sinks for intracellular calcium and thus have a vital role in regulating cytosolic calcium concentration. Bygrave (1976a) hypothesized that Ca^{2+} by inhibiting the transport of ADP into mitochondria was in a position to modify the energy metabolism of ascites tumour cells. Changes in calcium functions of mitochondria were observed under certain pathological conditions like dystrophy (Mezon *et al.*, 1974). In carbon tetrachloride and uranium nitrate intoxication (Carafoli *et al.*, 1971), physiological significance was attributed to these processes.

Preliminary experiments in this laboratory have shown that soon after exposure, the blood and tissue ionic levels particularly calcium increase (Indu Bashyam, unpublished results). Obviously to maintain intracellular homeostasis, mitochondria accumulate calcium.

Several observations on the mitochondrial behaviour reported by us could be explained by this hypothesis:

(i) The decreased ADP/O levels, respiratory control ratios, uncoupler stimulated respiration and ATPase levels could be due to the loaded-calcium. Uncoupling effect of calcium was also reported by other workers (Rossi and Lehninger, 1964; Hackenbrock and Caplan, 1969; Jacobus and Brierly, 1969; Thorne and Bygrave, 1974a,b; Bygrave, 1976a).

(ii) Influx of calcium can cause swelling (Rossi and Lehninger, 1964; Hackenbrock and Caplan, 1969). However, it should be mentioned that the swelling of the organelle under our experimental conditions could also be due to the hyperosmotic intracellular environment. It was suggested that the two opposing forces of electrophoretic cation uptake with osmotic swelling and exchange dependent

cation extrusion and contraction might represent a volume control mechanism for the mitochondrion within the cell which permitted the organelle to maintain its functional integrity in the presence of changes in the ionic environment (Borle, 1973).

Blair (1977) reported that Ca^{2+} loaded mitochondria did not show contraction responses to the addition of ATP and Mg^{2+} , which is very similar to the observations reported here. This could be either due to the uncoupled nature of the mitochondria, or competition between Ca^{2+} and Mg^{2+} for the sensitive sites. Ca^{2+} and Mg^{2+} have been shown to be competitive inhibitors for several ATPases and other enzymes (Lew, 1971; Blum and Hoffmann, 1972; Hackenbrock and Caplan, 1969; Bygrave, 1978; Mason and Tobes, 1977).

(iii) The decreased $^{45}Ca^{2+}$ uptake by calcium loaded mitochondria was demonstrated by Carafoli *et al.* (1971). This explains our earlier observation that mitochondria isolated from fish under stress had lowered capacity for Ca^{2+} uptake.

On the inter-relationship between sodium and calcium

Data given in figure 2 on the endogenous levels of ions, shows an inverse relationship between sodium and calcium. As calcium influxed into the mitochondria, sodium was effluxed out. It is interesting that similar effects have been observed under *in vitro* conditions also (table 1). Incubation of calcium-loaded mitochondria with sodium released the inhibited mitochindrial functions.

Carrier mediated exchange between Na^+ and Ca^{2+} is well documented in plasma membrane (Router, 1974),mitochondria from cardiac tissue, adrenal cortex and brain (Crompton *et al.*, 1976). In these tissues sodium induced a rapid efflux of Ca^{2+} ions. The recent results of Crompton *et al.* (1978) throw some doubt as to whether this recycling is present in skeletal muscle mitochondria. Although critical, quantitative studies are being carried out currently, the results presented in this paper show that muscle tissue may also possess such an Na^+ —Ca^{2+} antiport system.

On the changes in membrane composition

In our earlier study (Hannah Sulochana *et al.*, 1977b) we had presented physico-chemical evidence to suggest possible conformational changes in mitochondria. Recent analyses of the membrane composition show decreased cholesterol levels in the mitochondria under stressed conditions, conditions which also facilitate swelling of the organelle (Indu Bashyam, unpublished results). Decreased sterol levels could also affect the Mg^{2+} -ATPase activity (Graham and Green, 1970; Dianzani *et al.,* 1973; Feo *et al*., 1975; Astin and Haslam, 1977).

On the reversal of the process or adaptation

As has been mentioned, the various functions which were inhibited on the 8-9th day of stress were resorted to normal levels on continued exposure to stress by about the 21st day. At present, we do not have satisfactory explanations to present except to say that other physiological mechanisms (particularly hormonal) may have a role to play. Preliminary studies on this aspect with the gill tissue (Suresh Narayan, unpublished data) suggest the possibility that mitochondriogenesis may be occurring during this adaptive phase. This is under further investigation.

In analogy with the hypothesis of Rasmussen (1975) the presence of an ionic net in the cell that propagates and amplifies the original signal perceived by a membrane receptor, it can be proposed that each ionic compartment maintains its ionic composition at the expense of energy.

Acknowledgement

This research work was supported by a grant from the University Grants Commission, New Delhi.

References

Astin, A. M. and Haslam, J. M. (1977) *Biochem. J.,* **166,** 287.
Blair, P. V. (1977) *Arch. Biochem. Biophys.,* **181,** 550.
Blum, R. M. and Hoffman, J. F. (1972) *Biochem. Biophys. Res. Commun.,* **46,** 1146.
Borle, A. B. (1973) *Fed. Proc. Fed. Amerc. Soc. Expt. Biol.,* **32,**1944.
Bradford, M. M. (1976) *Anal. Biochem.,* **72,** 248.
Bygrave, F. L. (1976a) in *'An Introduction to Bio-inorganic Chemistry',* eds. D. R. Williams and C. C Thomas, Springfield, 111.,171.
Bygrave, F. L. (1976b) in *'Control Mechanisms in Cancer',* eds. W. E. Criss, T. Ono and J. R. Sabine, (New York: Raven Press, p. 411.
Bygrave, F. L. (1978) *Biol. Rev.,* **52**,43.
Carafoli, E. and Crompton, M. (1978) *Annal. N. Y. Acad. Sci.,* **307,** 269.
Carafoli, E. and Lehninger, A. L. (1971) *Biochem. J.,* **122,** 681.
Carafoli, E., Tiozzo, Pasquali-Ronchetti, I. and Laschi.R. (197I) *Laboratory Investigations,* **25**, 516.
Carafoli, E., Tiozzo, R., Lugli, G., Crovetti, F. and Kratzing, C. (1974) *J. Molec. Cell. Cardiol.,* **6,**361**.**
Crompton, M., Capano, M. and Carafoli, E. (1976) *Eur. J. Biochem.,* **69,** 453.
Crompton, M., Moser, R., Ludi, H. and Carafoli, E. (1978) *Eur. J. Biochem.,* **82,** 25.
Dianzani, F. F., Rita, G. A. and Zuretti, M. F. (1973) in *Future Trends in Inflammation',* eds. G. P. Vela, D. A. Willoughby and J. P. Giroud (Piccin Med. Books), 385.
Dow, S. D., Walton, K. G and Fleischer, S. (1976) *Bio energetics,* **1,** 247.
Epstein, F. H. and Wittam.R. (1966), *Biochem. J.,* **99,** 232.
Feo, F., Canuto, R.A., Garcea, R. and Gabriel, L. (1975) *Biochim. Biophys. Acta.,* **413,** 116.
Fiske, C. H. and Subbarow, Y. (1925) *J. Biol. Chem.,* **66,** 375.
Graham, J. M. and Green, C. (1970) *Eur. J. Biochem.,* **12,** 58.
Hackenbrock, C. R. and Caplan, A. I. (1969) *J. Cell. Biol.,* **42,** 221.
Hannah Sulochana, R., Honnappa, G. V. and Jayaraman, J. (1977a) *Indian J. Exptl. Biol.,* **15**,1102,
Hannah Sulochana, R., Indu Bashyam, Suresh Narayan and Jayaraman, J. (1977b) *J. Bioenerg. Biomem.,* **9,**337.
Honnappa, G. V., Hannah Sulochana, R. and Jayaraman, J. (1975) *Bioenergetics,* **7,**149.
Jacobus, W. E. and Brierly, G. P. (1969) *J. Biol. Chem.,* **244,** 2994.
Lehninger, A. L. (1959) *J. Biol. Chem.,* **234,** 2465.
Lehninger, A. L. (1965) in *'The Mitochondrion*: *Molecular Basis of Structure and Function',* (Amsterdam, W. A. Benjamin, Inc., New York), p. 164.
Lew, V. L. (1971) *Biochim. Biophys. Acta.,* **233,** 827.
Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randal, R. S. (1951) *J. Biol. Chem.,* **193,** 265.
Mason, M. and Tobes, M. C. (1977) *Biochem. Biophys. Res. Commun.,* **75,** 434.
Mezon, B. J., Wrogmann, K. and Blanchaer, M. (1974) *Can. J. Biochem.,* **52,** 1024.
Rasmussen, H. (1975) in *'CellularMembranes',* eds. G. Weissman, and Clairborne, R. (New York: H.P. Publishing Co. Inc.), p. 203.
Reuter, H. (1974) *Circ. Res.,* **34,** 3399.
Richardson, T. and Tappel, A. L. (1962) *J. Cell Biol.,* **13,**43.
Rossi, C. S., Carafoli, E., Drahota, Z. and Lehninger, A. L. (1966) in *'Regulation of Metabolic Processesin Mitochondria',* eds. J. M. Tager, S. Papa, E. Quagliariello and E. C. Slater (Amsterdam: Elsevier Publ. Co.) p. 239.
Rossi, C. S. and Lehninger, A. L. (1964) *J. Biol. Chem.,* **239,** 3971.
Thorne, R. F. W. and Bygrave, F. L. (1974a) *Biochem. J.,***144,** 551.
Thorne, R. F. W. and Bygrave, F. L. (1974b) *FEBS Lett.,* **41,**118.