ments shown in Fig. 1 was repeated except that [^{3}H]uridine was injected intracranially rather than in the eye, thus labeling only tectal cells and not optic axons (*13*).

Typical profiles of the radioactivity on 2 percent gels are shown in Fig. 2. With minor variations, radioactivity profiles were similar in all experiments and the proportion of radioactivity falling in each of the major stable RNA peaks was not altered by the experimental procedure. These results show that the selective loss of 4*S* RNA demonstrated earlier (Fig. 1) was probably caused not by changes in RNA metabolism in tectal cells, but by a loss of intraaxonal 4*S* RNA.

To determine the fraction of radioactivity present in the 4*S* peak, the total radioactivity on a 10 percent gel was determined and the portion falling in the 4*S* peak was compared with the total radioactivity on the gel for groups of fish injected either intraocularly or intracranially. After intraocular injection, greater than 50 percent of the radioactivity was associated with 4*S* RNA regardless of whether that radioactivity was measured 6 days (53 percent) or 12 days (67 percent) after injection. However, after degeneration of the optic nerve only about 21 percent of the radioactivity was associated with 4*S* RNA. After intracranial injections, the proportion of radioactivity in the 4*S* peak was approximately 25 percent whether optic axons were present or not.

These results, considered with those of earlier studies (*4*–*7*), strongly indicate that a large portion of 4*S* [^{3}H]RNA present in goldfish optic tecta during optic nerve regeneration and after intraocular injection of [^{3}H]uridine is due to the presence of 4*S* [^{3}H]RNA in regenerating optic axons. It is likely that this RNA is synthesized in retinal ganglion cells and then axonally transported in the growing optic axons.

Some evidence suggests that small amounts of RNA are transported in normal optic axons (*4*), but we have not been able to confirm this by autoradiographic or biochemical studies. Therefore, the question of whether RNA is axonally transported in nongrowing optic axons of goldfish remains unanswered. It also remains to be seen if the 4*S* RNA in goldfish optic axons is transfer RNA, as has been shown for squid axons (*3*). Other experiments indicate that 4*S* RNA is transported axonally in the chick visual system (*14*), in both normal and regenerating rat sciatic nerves (*15*), and in optic axons of neonatal rats (*16*). The observation that 4*S* RNA is the only RNA species found in a variety of axons may require a reappraisal of the function of 4*S* RNA in nervous tissue. It may be that the 4*S* RNA is transfer RNA and either donates an amino acid to existing axonal polypeptides, thus modifying axonal proteins (*3*), or is transferred to surrounding tectal cells where it may participate in or regulate protein synthesis (*15*). Alternatively, axonal 4*S* RNA may serve a function in growing axons separate from its role in protein synthesis, a function yet to be described.

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References and Notes

1. A. Hughes and L. B. Flexner, *J. Anat.* **90**, 386 (1956); A. Edstrom, *J. Neurochem.* **11**, 309 (1964); E. Koenig, *ibid.* **12**, 357 (1965); R. J. Lasek, *ibid.* **17**, 103 (1970); P. Gambetti, L. Autilio-Gambetti, B. Shafer, L. Pfaff, *J. Cell Biol.* **59**, 677 (1973).
2. R. J. Lasek, C. Dabrowski, R. Nordlander, *Nature (London) New Biol.* **244**, 162 (1973).
3. M. M. Black and R. J. Lasek, *J. Neurobiol.* **8**, 229 (1977).
4. N. A. Ingoglia, P. Weis, J. Mycek, *ibid.* **6**, 549 (1975).
5. P. Gambetti, N. A. Ingoglia, L. Autilio-Gambetti, P. Weis, *Brain Res.* **154**, 285 (1978).
6. N. A. Ingoglia and R. Tuliszewski, *ibid.* **112**, 371 (1976).
7. N. A. Ingoglia, *J. Neurochem.* **30**, 1029 (1978).
8. Optic nerves were crushed by first submerging goldfish (*Carassius auratus*), 10 to 13 cm in length (Millbrook Farms, Upper Saddle River, N.J.), in ice water for 10 minutes. Optic nerves were then exposed in the anesthesized fish and crushed by compression with curved jewelers' forceps.
9. Details of the extraction procedure and methods for recovering radioactivity from gel slices are given in (*6*).
10. Electron microscope studies of the goldfish optic tectum 6 days after optic nerve section have shown marked degenerative changes in retinal fibers [M. Murray, *J. Comp. Neurol.* **168**, 175 (1976)]. In other studies we have shown that 6 days after optic nerve section 95 percent of rapidly axonally transported proteins are lost from the tectum [N. A. Ingoglia, B. Grafstein, B. McEwen, I. McQuarrie, *J. Neurochem.* **20**, 1605 (1973)]. Thus it appears that a large portion of the contents of optic axons in the tectum are removed from the tectum within a week of cutting the optic nerve.
11. The procedures followed were essentially those of U. E. Loening [*Methods Med. Res.* **12**, 359 (1970)] for 2 percent gels and of K. Weber and M. Osborn [*J. Biol. Chem.* **244**, 4406 (1969)] for 10 percent gels.
12. While Fig. 1 shows relative changes in radioactivity in each of the RNA fractions, there is also a clear decrease in the amount of radioactivity in the 4*S* RNA peak. The actual disintegrations per minute associated with each of the RNA peaks are as follows. Group 1: 28*S*, 1348; 18*S*, 855; and 4*S*, 3647. Group 2: 28*S*, 6424; 18*S*, 4300; and 4*S*, 1741. Group 3: 28*S*, 4984; 18*S*, 2525; and 4*S*, 17,022.
13. Both optic nerves in 36 fish were crushed, and 18 days later 8 μCi of [^{3}H]uridine was injected intracranially. Four days later, one group of 12 fish was killed and the optic nerves of a second group of 12 were cut. The second group and a third group of 12 fish were then killed 11 days after injection. The RNA was extracted from tecta of all three groups as described above and layered on 2 and 10 percent SDS-polyacrylamide gels.
14. S. Por, P. W. Gunning, P. L. Jeffrey, L. Austin, *Neurochem. Res.* **3**, 441 (1978).
15. T. D. Lindquist and N. A. Ingoglia, *Brain Res.* **166**, 95 (1979).
16. M. Politis and N. A. Ingoglia, *ibid.* **169**, 343 (1979).
17. This work was supported by a grant from the Foundation of the College of Medicine and Dentistry of New Jersey and by grant EI-02887 from the National Institutes of Health.

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Shark Heart Mitochondria: Effects of External Osmolality on Respiration

Abstract. *Shark mitochondrial respiration was studied in media with osmolalities between 160 and 1500 milliosmoles. The respiratory control ratio, a marker for functional integrity of the isolated mitochondria, was maximal at 1000 milliosmoles and decreased during hypotonic or hypertonic exposure. Shark mitochondria function best at their native tonicity, a value that produces abnormal function in mammalian mitochondria.*

Isolated mammalian mitochondria show striking changes in respiratory control when osmolalities of suspending media are less (hypotonic) or greater (hypertonic) than the normal value of approximately 300 mOsm (*1*, *2*). In the studies reported here we measured respiratory control ratios (RCR) and adenosine diphosphate/oxygen (ADP/O) ratios in isolated shark heart mitochondria exposed to external osmolalities varying from 160 to 1500 mOsm. We found that respiratory control is maximal at 1000 mOsm, the usual osmolality in shark cells. This suggests that shark mitochondria are functionally adapted to an osmolality considerably different from that of mammalian mitochondria.

Pelagic sharks approximately 1 m long, indigenous to the San Diego Bay area (*3*) were killed by decapitation. The heart was minced in isolation medium (*4*) and homogenized by hand in a Ten Broeck glass tissue homogenizer. The suspension was centrifuged at 1000*g* for 2 minutes and the resultant supernatant at 17,000*g* for 5 minutes. The final pellet was suspended in isolation medium to a concentration of approximately 10 mg of protein per milliliter.

Mitochondrial oxygen consumption ($\dot{Q}O_2$) was determined polarographically at 25°C with a Gilson oxygraph and Clark platinum electrode assembly, as described in (*1*). Heart mitochondria were added to the reaction chamber, which contained the incubation medium of the experimental osmolality (*4*) and the sub-

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Table 1. Values of $\dot{Q}O_2$ of isolated shark heart mitochondria at various osmolalities in the presence (state III) and absence (state IV) of limiting amounts of ADP (mean ± standard error). Units are micromoles of O_2 consumed per hour per milligram of mitochondrial protein. Also shown is ADP/O, the ratio of micromoles of ADP added to microatom equivalents of O_2 consumed; N, number of studies.

Osmolality (mOsm)	$\dot{Q}O_2$ (μM/hour-mg)		ADP/O	N
	State III	State IV		
160	3.35 ± 0.95*	1.12 ± 0.27*	2.5 ± 0.2	4
300	2.90 ± 0.42	0.63 ± 0.09*	2.7 ± 0.2	8
600	2.65 ± 0.25	0.54 ± 0.07*	2.5 ± 0.2	7
1000	2.31 ± 0.24	0.38 ± 0.05	2.5 ± 0.2	8
1500	1.38 ± 0.36*	0.48 ± 0.08	1.8 ± 0.6	4

*Significantly different from the mean at 1000 mOsm at $P < .05$ (paired t-test).

strate for the initiation of electron transfer (glutamate and malate, 1.25 mM each) (*5*). After 5 minutes of incubation, ADP was added to the chamber to a concentration of 90 μM (*6*). The increase in $\dot{Q}O_2$ as the ADP was phosphorylated to adenosine triphosphate was designated as state III and the resumption of baseline $\dot{Q}O_2$ after the ADP was consumed was designated as state IV (*7*). Protein concentration of the mitochondrial suspension was determined by the method of Lowry *et al.* (*8*) and was usually 1.0 mg/ml. The results for each experimental osmolality are tabulated in Table 1. Mean $\dot{Q}O_2$ for the experimental osmolalities were compared to values obtained at 1000 mOsm by paired t-test. The RCR was defined as the ratio of state III to state IV or the relative increase in $\dot{Q}O_2$ during oxidative phosphorylation (*9*). Values of RCR for shark mitochondria at different osmolalities are compared to values for rat liver mitochondria (*1*) in Fig. 1. The ADP/O ratios are tabulated for each osmolality in Table 1.

The highest RCR was seen at 1000 mOsm with lower ratios as osmolality varied in either direction (Fig. 1). Mitochondria incubated in hypotonic media showed an increase in both state III and state IV with a greater percentage increase in the latter (Table 1). Mitochondria incubated in media hypertonic to the tissue showed maintenance of state IV respiration but suppression of state III. The ADP/O ratios remained nearly constant with hypotonic exposure but decreased during hypertonic exposure, a phenomenon also seen in rat liver mitochondria (*1*).

Respiratory control has been a useful probe of the functional integrity of isolated mitochondria in a variety of experimental conditions. Although the physiological significance of this term has not been correlated with the energetics of the intact cell, rapid utilization of O_2 for oxidative phosphorylation (high state III) and minimal utilization of O_2 for nonphosphorylation-coupled functions (low state IV) are considered biologically useful. Maximal respiratory control is seen in two widely divergent species, rats and sharks, at the native osmolality of the tissue. Changes in respiratory control with hypotonic or hypertonic exposure of shark mitochondria are similar to those seen for rat liver mitochondria but are shifted some 700 mOsm (Fig. 1). Although we have reported data only for rat liver mitochondria, similar changes are reported in mammalian heart and kidney mitochondria (*10*). Thus osmotic optima around 300 mOsm appear to be characteristic of mammalian mitochondria.

Preservation of the ADP/O ratio of the shark mitochondria during hypotonic incubation suggests that the thermodynamic efficiency of the organelle is still intact even though the respiratory control mechanisms appear to have been affected (*9*). The basic mechanisms by which altered osmolality alters mitochondrial function are not known in terms of the Mitchell chemiosmotic hypothesis for oxidative phosphorylation (*11*). Since basal O_2 consumption (state IV) was not reduced, we speculate that altered osmolality interferes with the development of an adequate H^+ gradient across the membrane or that O_2 is diverted from utilization for the production of the chemiosmotic gradient itself. The observations of Kryvi and Slinde (*12*) that shark muscle mitochondria maintain microstructural integrity over a broad range of extramitochondrial osmolalities do not resolve this since it is well known that functional abnormalities may be demonstrated in the absence of ultrastructural changes.

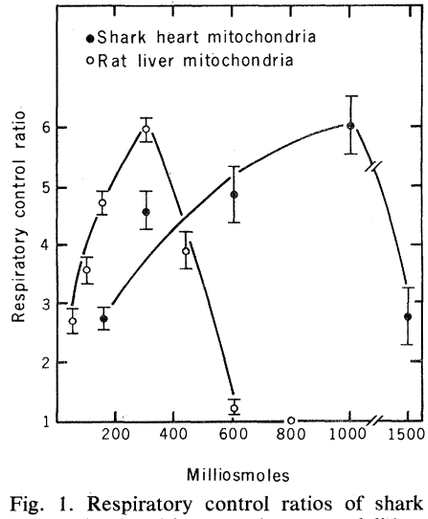

Fig. 1. Respiratory control ratios of shark heart mitochondria at various osmolalities. Ratios for rat liver mitochondria treated in the same fashion (*1*) are shown for comparison. Error bars designate the standard error.

The effects of osmolar stress were identical in the several species of sharks that we studied. These results suggest that the evolution of shark mitochondria has paralleled the development of relatively concentrated body fluids with a high urea content, providing optimal function at a cellular tonicity substantially greater than that of other vertebrates.

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References and Notes

1. D. Holtzman, N. Lewiston, M. Herman, M. Desautel, E. Brewer, E. Robin, *J. Neurochem.* **30**, 1409 (1978).
2. M. Barrett and A. Horton, *Biochem. Soc. Trans.* **4**, 64 (1976); M. Matlib and P. Srere, *Arch. Biochem. Biophys.* **174**, 705 (1976); D. Johnson and H. Lardy, *Nature (London)* **181**, 701 (1958).
3. Leopard shark, *Triakis semifasciata*; smooth hound, *Mustelus mustelus*; swell shark, *Cephaloscyllium uter*; horned shark, *Heterodontus francisci*.
4. Isolation medium was 400 mM mannitol, 400 mM sucrose, 150 mM KCl, 0.2 mM EDTA, and 5 mM tris-HCl, pH 7.4. Media of experimental osmolalities were prepared by varying the content of sucrose and mannitol. The osmolality of each solution was verified with a Fiske osmometer (Fiske Associates, Inc., Bethel, Conn.).
5. Reagent grade glutamic acid, malic acid, and ADP were obtained from Sigma Chemical Co., St. Louis, Mo.
6. B. Chance and G. Williams, *Nature (London)* **175**, 1120 (1955).
7. B. Chance and M. Baltscheffsky, *Biochem. J.* **68**, 283 (1958).
8. O. Lowry, N. Rosebrough, A. Farr, J. Lampen, *ibid.* **71**, 331 (1959).
9. B. Chance, in *Ciba Foundation Symposium on the Regulation of Cell Metabolism*, G. Wolstenholme and C. O'Connor, Eds. (Little, Brown, Boston, 1959), p. 91.
10. E. Slater and K. Cleland, *Biochem. J.* **53**, 557 (1953); A. Atsmon and R. Davis, *Biochim. Biophys. Acta* **131**, 221 (1967).
11. P. Mitchell, *Biol. Rev.* **41**, 445 (1965).
12. H. Kryvi and E. Slinde, *Cytobiologie Z. Exp. Zellforsch.* **15**, 221 (1977).

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