Changes in membrane phosphorylated proteins (*13*) and cell-surface antigens (*14*) during FLC differentiation have been reported. Pools of phosphorylcholine have been observed in Chinese hamster ovary cells (*15*).

Thus, ^{1}H spin echo NMR can be used to observe specific changes in metabolite levels in both intact cells and extracts during a controlled experiment such as the induced FLC differentiation. Methods described here have been easily adapted to the study of antimycin-resistant and -sensitive strains of cells grown in monolayer cultures (*16*). Whole cell ^{1}H NMR allows simultaneous monitoring of many cytoplasmic compounds at low concentrations in H_2O. The methods are applicable to lower field (300 MHz) instruments when cells are washed in D_2O medium or solvent suppression is used. Cytoplasmic extracts can be useful as tools for the assignment of signals as well as sources of substrates, competitors, and inhibitors of enzymes, whose activities can be assayed by ^{1}H NMR.

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A Brain Heater in the Swordfish

Abstract. *The brain and eye of swordfish are warmer than the water. Associated with one of the eye muscles is a tissue that heats the brain. This brain heater is rich in mitochondria and cytochrome c and is supplied with blood through a vascular heat exchanger. It protects the central nervous system from rapid cooling during daily vertical excursions which may take the swordfish through a wide temperature range.*

Swordfish, *Xiphias gladius*, are large pelagic fish that range over the world's temperate and tropical oceans. The preponderance of white fibers in their swimming muscle indicates that they are stalkers and sprinters (*1*). They do not maintain the high level of continuous activity that we associate with warm fishes with elevated body temperatures such as the tunas (*2*) and, indeed, their muscle and viscera are close to water temperature (*3*). Swordfish are creatures of semidarkness, spending the night near the surface, but going as deep as 600 m during the day (*4*). In these vertical excursions water temperature may change as much as 19°C in less than 2 hours (*4*). The large and abrupt temperature changes that swordfish experience daily would chill the brain and affect central nervous system processes in most fish (*5*), but swordfish have developed a heater, which warms the brain and eye. This mass of specialized tissue and its associated vascular heat exchanger warm these organs to temperatures significantly above that of the surrounding water and reduce the extent of temperature fluctuations.

Associated with one of the eye muscles, the rectus superior, is a large swelling closely applied to the ventral side of the brain case. The tissue in this structure is brown, with the color and consistency of liver. Its blood supply is by way of a highly developed rete mirabile that arises from the carotid artery and forms a dense mass of small (80 to 100 μm in diameter), parallel arteries and veins. The rete is large for the mass of tissue it serves: in a 120-kg swordfish, the rete leading to a 50-g mass of brown tissue and muscle has a cross section area of 2 cm^2. As it merges with the brown tissue, the rete divides into strands of parallel vessels and blood is delivered to the surrounding brown tissue cells by a radiating pattern of sinusoids that have some resemblance to those of liver. The brown tissue cells are cuboidal with dense brown cytoplasm and clear distinct nuclei. They are packed with mitochondria and contain numerous small vacuoles. The distinctive brown color of the tissue is due to its high concentration of cytochromes. Cytochrome c concentration was 35 ± 3 nmole/g (*6*), similar to the 22 to 33 nmole/g reported for the brown fat of various small mammals (*7*). The ample blood supply, numerous mitochondria, and high cytochrome c content are similar to mammalian brown fat and indicate an unusually high metabolic rate in the brown tissue.

The brain of swordfish is warm (*8*). For fish caught on longline fishing gear (*9*), the brain, eye, and brown tissue are significantly warmer than the water (Table 1). Because swordfish captured on longline are usually dead or in poor condition, temperatures of undisturbed fish are probably higher than those shown in Table 1 (*10*). The highest temperatures yet recorded in swordfish were obtained from a free-swimming fish in an acoustic telemetry experiment where during a 36-hour period temperature in the cranial cavity was 10° to 14°C warmer than the water (*4, 11*).

The temperatures of fishes are tightly coupled to water temperature by circulation of the blood, which acts as a convective cooling system. Metabolic heat produced in the tissues is carried away by the blood and lost to the environment through the gills (*12*) so that fish remain close to water temperature. Warm fishes have developed countercurrent heat exchangers in their circulatory system and these retain metabolic heat and raise temperatures (*13*). The large rete mirabile serving the swordfish brown tissue is such a countercurrent heat exchanger. The venous and arterial flow in the retial vessels is in opposite directions and the alternating arrangement of the tightly

Table 1. Temperatures in billfish heads; *N* is indicated in parentheses.

Fish	Water (°C)	Temperatures (°C above water)		
		Retina	Brain	Brown tissue
Swordfish	19.8 ± 3.4 (11)	3.4 ± 1.7 (8)	4.7 ± 2.0 (9)	4.3 ± 2.0 (11)
White marlin	20.9 ± 1.8 (4)			3.4 ± 1.1 (4)
Sailfish	25.6		3.2	1.1

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packed, 0.1-mm-diameter arteries and veins is appropriate for heat exchange. Temperature measured along the rete increases from that of water at the end of the rete near the carotid artery to that of the brain at the brown tissue end (Table 2) (Fig. 1). The presence of such a temperature gradient identifies the rete as the site where heat is transferred from the venous to the arterial stream, conserving metabolic heat within the brown tissue and elevating its temperature.

The brain of swordfish, like that of other large teleosts, is small for its body size. (In a 136-kg swordfish the brain weighed 2.2 g, or 0.002 percent of the body weight.) The brain is such a small mass of tissue that even with an efficient heat exchanger in its circulation, it probably could not maintain a significant temperature elevation in an otherwise cold head. The brain heaters consist of a specialized mass of thermogenic tissue 50 times as heavy as the brain (150 g in the 136-kg swordfish), and they produce enough heat to warm the adjacent brain. Unlike mammalian brown fat where blood flow spreads the heat through the body, the rete serving the brown tissue prevents such convective dissipation and confines the heat to the vicinity of the brain.

The cross-section view of the swordfish head in Fig. 1 shows that the brain is well placed to be warmed by conduction of heat from the brown tissue. A portion of the basisphenoid bone that forms the floor of the cranial cavity is uncalcified in swordfish (*14*) and is present only as a thin (0.5 mm) connective tissue membrane beneath the brain. The brain lies in a V-shaped depression in this membrane, partially embedded in the brown tissue, which is closely applied to its bottom and sides. The space beneath the brain case is taken up by the eye, its muscles, and a large amount of orbital fat. The brain is near the center of the head; above, it is covered by a layer of white fat (5 to 10 cm thick) and fatty bone; laterally, the brain and brown tissue are shielded by the thick mass of fat and eye muscles. The thermal conductivity of fat is only about one-third that of water (*15*), and the brain and brown tissue are thus insulated from loss of heat through conduction to the surface of the head.

Table 2. Temperature gradient measured at positions indicated in the legend of Fig. 1 for swordfish. The same numbers are used here for the corresponding positions in the white marlin.

1	2	3	4
Swordfish			
1.3	2.5	3.5	2.5
0.9	3.2	5.0	4.6
−0.8	1.3	4.8	8.5
White marlin			
1.3	2.1	2.8	2.7

Since the brain is well supplied with blood, convective heat transfer by the circulation is potentially an important route of heat loss. Without a heat exchange system in its circulation, the brain would be cooled by cold arterial blood from the gills. There appear to be several ways for providing warm arterial blood for the swordfish brain. The encephalic artery, a major artery supplying the brain in most fish, went unnoticed in dissections of the swordfish head and, if present, is a small vessel. There are, however, several small arteries of the carotid rete serving the brown tissue and these continue dorsally beyond the brown tissue and enter the cranial cavity through a foramen with the III, V, and VII nerves. Inside the cranial cavity, these arteries join a plexus around the brain. Blood arriving by this route would already be warmed by passing around the brown tissue. A more superficial route of blood flow to the brain is through the hyoopercularis artery on the side of the head. This artery passes between the abductor mandibularis and the levator arcus palatini muscles where it and the accompanying veins are elaborated into a flattened rete mirabile. Some branches of this rete go to the muscles, but a major branch reforms into a single vessel which enters the cranial cavity through a foramen in the sphenotic bone. At the rear of the cranial cavity this artery joins a plexus associated with the auditory apparatus, and from this plexus branches run ventrally along the sloping walls of the cranial cavity to the brain. Blood flowing to the brain by this route would be warmed by passage through the jaw muscle rete and by heat from the brown tissue during its course down the wall of cranial cavity.

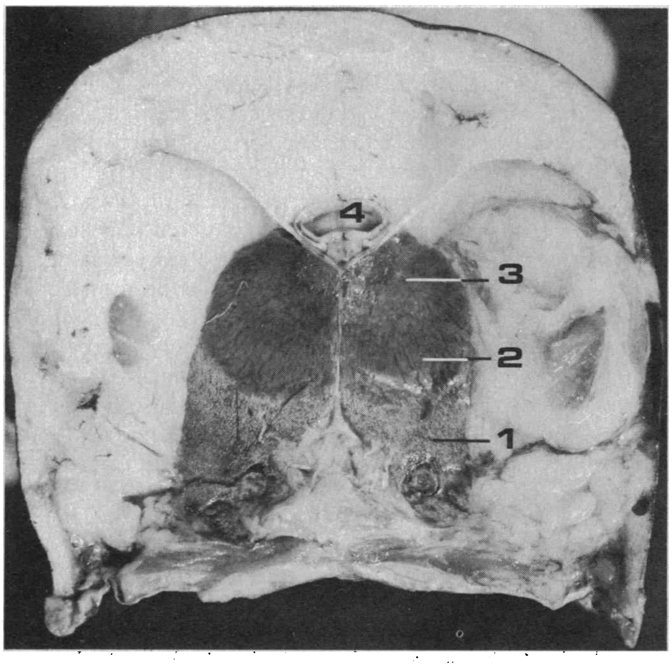

Fig. 1. A cross section through the head of a small (14 kg) swordfish at a level just behind the eyes. The brain, labeled "4," is seen in the center, nested into the brown tissue, but separated from it by the membranous remnant of the basisphenoid bone. Merging into the brown tissue from below is the carotid rete which has a fibrous appearance from injection of latex into the arterial vessels. The circular form near the base of the rete is a branch of retial vessels which surrounds the ophthalmic major artery on its course to the eye. Large amounts of solid white fat behind the eyes and a 36-mm-thick layer above the brain insulate the brain and brain heater. The approximate locations of temperatures given in Table 2 are indicated as: *1*, in the rete near the carotid artery; *2*, near the fusion of the rete with the brown tissue; *3*, in the brown tissue near the brain; and *4*, the brain.

Cold has a pronounced effect on the integrative functions of the nervous system. In many organisms a rapid drop in temperature will delay responses and interfere with learned behavior (*5*). The swordfish is an active predator that strikes and seizes swiftly swimming prey (*16*). The brain heater is probably important in allowing the fish to hunt effectively during the large temperature changes which are part of its daily routine. Quality in a central nervous system is commonly equated with a large brain, but apparently the swordfish, with a brain only 0.002 percent of its body weight and only 1/50th the weight of the organ which warms it, is responding to requirements other than those of intellect.

Brain heaters occur in species other than swordfish. White marlin (*Tetrapturus albidus*) and sailfish (*Istiophorus platypterus*) have retia and lumps of brown tissue at the base of the rectus superior eye muscle, and brain and brown tissue temperature elevations of 3° to 4°C have been recorded from these fish (Table 1). Other billfishes—blue marlin (*Makaira nigricans*), striped marlin (*Tetrapturus audax*), and shortbilled spearfish (*Tetrapturus angustirostris*)—have such swellings of brown tissue on the rectus superior muscle and will probably also be found to have warm brains. These fish, particularly the sailfish and spearfish, are thought to be surface dwellers of warm tropical seas. The utility of a heating system for the brain is not apparent in this environment, but little is known about the natural history and behavior of these fishes and it can only be assumed that there are situations where it is of advantage to them.

Gasteroschisma melampus (family Scombridae) is a large pelagic fish of the Southern Ocean, which also appears to have a brain heater. No temperature information is available from this fish, but it does have a mass of brown tissue associated with one of its eye muscles. As in the billfishes, the brown tissue is supplied with blood through a large rete. In *Gasteroschisma*, however, it is the rectus posterior rather than the rectus superior eye muscle that has developed this specialization. The evolution of similar brain heaters from different eye muscles in billfishes and in *Gasteroschisma* indicates that eye muscle is particularly suitable for serving as a source of heat for the brain (*17*). Most pelagic fish are active visual predators. The eye muscles of tuna and billfish have a red color (*18*) from an abundance of red muscle fibers, an indication that the eyes are in motion much of the time. The presence of such active tissue, well insulated with fat and located close to the brain, may have provided the opportunity for evolution of the brain heater.

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Biotin Enhances Guanylate Cyclase Activity

Abstract. *Biotin and its analog, (+)-biotin-*p*-nitrophenyl ester enhanced guanylate cyclase activity two- to threefold in rat liver, kidney, colon, cerebellum, and heart. Dose-response relationships revealed that at concentrations as low as 1 micromolar, both biotin and its analog caused maximal augmentation of guanylate cyclase activity. These data suggest a role for the activation of guanylate cyclase in the mechanism of action of this vitamin.*

Biotin, a growth-promoting factor at the cellular level (*1*), increases RNA (*2*) and protein synthesis (*3*). The mechanism by which biotin causes these effects is unknown. Since guanosine 3′,5′-monophosphate (cyclic GMP) increases the growth of fibroblasts (*4*) and thymocytes (*5*) and also increases RNA (*6*) and protein synthesis (*7*), the present investigation was conducted to determine whether biotin's effects might be mediated by cyclic GMP. We found that biotin increased cyclic GMP levels twofold in rat liver, kidney, colon, cerebellum, and heart. Further investigation revealed that the cause of these increased cyclic GMP levels was enhancement of soluble guanylate cyclase (E.C. 4.6.1.2) activity. An analog of biotin, (+)-biotin-*p*-nitrophenyl ester, increased guanylate cyclase activity to a similar extent (two- to threefold).

Tissues from Sprague-Dawley rats were processed (*8*) to obtain the 37,000*g* supernatant and particulate fractions. Guanylate cyclase was assayed as previously described (*8*) with a reaction mixture consisting of 20 m*M* tris-HCl, (*p*H 7.6), 4 m*M* $MnCl_2$, 2.67 m*M* cyclic GMP (used to minimize destruction of ^{32}P-labeled cyclic GMP), a guanosine triphosphate (GTP) regenerating system consisting of 5 m*M* creatine phosphate, 11.25 U of creatine phosphokinase (E.C. 2.7.3.2), 100 μg of bovine serum albumin, 20 m*M* caffeine, and 1.2 m*M* [α-^{32}P]GTP (approximately 5×10^5 count/min). The enzyme preparations had 0.1 to 0.2 mg of protein. The cyclic [^{32}P]GMP formed was isolated by sequential chromatography on Dowex-50-H^+ and alumina (*8*). Reactions were conducted at 37°C. (+)-Biotin and (+)-biotin-*p*-nitrophenyl ester were obtained from Sigma Chemical Company, St. Louis, Missouri. The sources of all of the other reagent-grade reagents have been reported (*8*). Each assay was conducted in triplicate, and the results were confirmed in three animals in each group in each of three separate experiments. Cyclic GMP tissue levels were measured by radioimmunoassay (*9*).

Biotin and (+)-biotin-*p*-nitrophenyl ester enhanced soluble guanylate cyclase activity in a variety of tissues (Table 1). Thus, the analog and biotin itself enhanced guanylate cyclase activity two- to threefold in rat liver, kidney, colon, cerebellum, and heart. Both agents in-