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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8. Temperatures were measured by cutting off the head perpendicular to the body axis a short distance behind the eyes (Fig. 1) and probing the exposed tissues with a thermistor mounted in 18-gauge steel tubing. The *t*-test for paired comparisons [R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969), p. 331] showed that the swordfish tissues were significantly warmer than the water at greater than the .001 level and the marlin at greater than the .01 level.
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11. The telemetry system was similar to that described in (*4*), but the transmitters had thermistors mounted in the ends of 30-cm lengths of waterproof cable (2.4 mm in diameter). Using features on the surface of the head for reference, a 2.5-mm hole was made to one side of the center line at the level of the brain, and the thermistor was pushed into a position near, but not in the brain. During the 36-hour experiment, water temperature fluctuated from 14.5° to 17°C and cranial temperature from 27° to 29°C. In a second telemetry experiment the cranial temperature was 19°C in water at 8°C.
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19. I thank Dr. J. Kanwisher, Woods Hole Oceanographic Institution, for design of the acoustic telemetry system; Chief Scientist Andrzej Majewicz and the officers and crew of the research vessel *Wieczno*, Morski Instytut Rybacki, Gdynia, Poland, for use of that vessel in the telemetry experiments; Captain James Ruhle and crew of the fishing vessel *Doranna R*, who provided the live swordfish in healthy condition; Dr. W. J. Richards, NOAA National Marine Fisheries Service Miami Laboratory, for encouragement to inspect his specimen of *Gasteroschisma*; and B. Block, Duke University, for discussions of circulatory anatomy and swordfish temperatures. Supported by NSF grants BMS-7306942, PCM-7681612 and OCE-8018674 and by contracts from the National Marine Fisheries Service. Contribution No. 5019 from the Woods Hole Oceanographic Institution.

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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-

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Table 1. The effect of biotin and its analog (+)-biotin-*p*-nitrophenyl ester on soluble guanylate cyclase activity of various tissues. The supernatant of each of the respective tissues was assayed as described in the text. Each value represents the mean ± standard error of the mean (S.E.M.) of triplicate samples determined in three separate experiments, with three animals for each experiment. Biotin and (+)-biotin-*p*-nitrophenyl ester were tested at their 1 μ*M* concentrations.

Tissue	Cyclic GMP (pmole per milligram of protein per 10 minutes)		
	No addition	Biotin*	(+)-Biotin-*p*-nitrophenyl ester*
Liver	283 ± 10	592 ± 22	671 ± 33
Colon	588 ± 23	1157 ± 31	1239 ± 29
Kidney	292 ± 14	889 ± 29	988 ± 24
Cerebellum	477 ± 17	968 ± 16	997 ± 21
Heart	163 ± 11	401 ± 19	603 ± 30

*Significant at $P < .001$ compared to control by Student's *t*-test for unpaired values.

creased cyclic GMP levels twofold in tissue slices of these same tissues (data not shown). Dose-response relationships indicated that both biotin and (+)-biotin-*p*-nitrophenyl ester enhanced guanylate cyclase activity to a similar extent, although the augmentation was always somewhat less with biotin (Fig. 1). Half-maximal stimulation of guanylate cyclase activity occurred at 0.1 μ*M* with biotin and (+)-biotin-*p*-nitrophenyl ester. Both agents caused their maximal enhancement of guanylate cyclase activity at their 1 μ*M* concentrations. Increasing the concentration to the millimolar range for either agent caused no further augmentation of guanylate cyclase activity. Similar dose-response curves were seen with the other tissues utilized. At 37,000*g*, particulate guanylate cyclase activity makes up only 5 percent of the total guanylate cyclase activity. There was very little effect of biotin or (+)-biotin-*p*-nitrophenyl ester on particulate guanylate cyclase activity (data not shown).

Biotin and (+)-biotin-*p*-nitrophenyl ester enhanced guanylate cyclase activity in vitro at or below concentrations of biotin that increase RNA (*2*) and protein synthesis (*3*). Biotin is a growth-promoting factor (*1*), and its mechanism of action seems similar to that of other growth-promoting factors, such as growth hormone (*8*), gibberellic acid (*10*), and fibroblast growth factor (*4*), all of which enhance guanylate cyclase activity to a similar extent. The present findings appear pertinent to earlier investigations that have suggested that guanylate cyclase and its product cyclic GMP are important in cell growth (*11*). Biotin is the first vitamin demonstrated to increase guanylate cyclase activity. Whether other vitamins will likewise augment guanylate cyclase activity awaits further investigation.

Insight into how biotin interacts with the enzyme guanylate cyclase itself may be gained from biotin's structural formula and the known function of biotin as a cofactor in carboxylation and transcarboxylation reactions. Biotin is an unstable compound that easily undergoes decarboxylation to produce a carboxylate anion. Since carbonyl anion (*12*), as well as another anion (superoxide, O_2^-) (*13*), has been previously implicated in enhancing guanylate cyclase activity, it is possible that the actual mechanism by which biotin augments guanylate cyclase activity is through an interaction of carboxylate anion with guanylate cyclase. The activation of guanylate cyclase by (+)-biotin-*p*-nitrophenyl ester may also be through carboxylate anion interaction with guanylate cyclase. This biotin analog, however, has another possible interaction with guanylate cyclase in that this compound is capable of generating a potential nitric oxide radical. Nitric oxide itself can enhance guanylate cyclase activity (*14*). A group of atoms that carries an unpaired electron and that is extremely reactive is designated a free radical. Free radicals as the actual final activator of guanylate cyclase have been suggested for compounds capable of generating nitric oxide (*14*) and hydroxyl radicals (*15*). The compounds that produce nitric oxide radicals have been, in general, the most potent stimulators of guanylate cyclase activity (*16*), and this may be the reason that (+)-biotin-*p*-nitrophenyl ester was found to be a more potent enhancer of guanylate cyclase activity than biotin in the present investigation. Another possible explanation of the greater increases in guanylate cyclase activity observed with (+)-biotin-*p*-nitrophenyl ester is that this analog contains both a potential carboxylate anion and a potential nitric oxide radical, and the observed effects may have been the result of an additive effect on this enzyme.

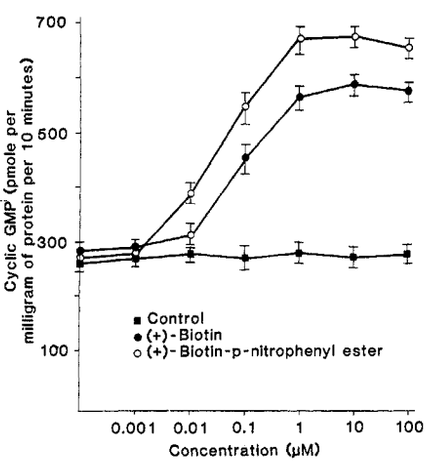

Fig. 1. Dose-response relationships of (+)-biotin and (+)-biotin-*p*-nitrophenyl ester on hepatic guanylate cyclase activity in vitro. Each value is the mean ± S.E.M. of triplicate samples determined in three separate experiments with three animals for each experiment ($N = 9$). The value at 0.01 μ*M* for (+)-biotin-*p*-nitrophenyl ester and at 0.1 μ*M* for biotin were significant at $P < .001$ compared to control by Student's *t*-test for unpaired values.

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