mum required weight is attained. However, this does not negate the finding that a critical minimum weight appears to be necessary for the onset and maintenance of normal menstrual cycles in the human female.

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Toxicity in Sponges and Holothurians: A Geographic Pattern

Abstract. *Toxicity in sponges and holothurians is inversely related to latitude and may reach 100 percent for holothurians in high-diversity coral reefs. Evidence from approximately 700 experiments and from underwater observations suggests that predation by fish has resulted in natural selection for noxious and toxic chemical compounds in species within these taxa.*

A decade ago it was suggested that toxicity is one of several defense mechanisms that benthic marine invertebrates have evolved in response to predation and grazing by coral reef fish (*1*). Further information has appeared on toxicity in coral reef benthic invertebrates [see references in (*2*, *3*)]. McAllister (*4*) stated that only 12 of 770 species of Canadian freshwater and marine fish are toxic to man and most of these are rare. Seven of the 12 toxic species are venomous. In contrast, data from Halstead and Mitchell and from Halstead [cited in (*2*, *3*)], suggested that both venomous and poisonous fish are considerably more common in the tropics than outside the tropics. The data for other marine taxa are insufficient for such a comparison. Because adequate information on toxicity has been unavailable for specific geographic sites, we now report on the extent to which toxicity in sponges and holothurians may change with latitude and with related water temperature and habitat.

Earlier studies (*5*) indicated that a variety of marine fish are sensitive to steroid saponins (holothurin) from certain holothurians and that, although freshwater fish are slightly more resistant to holothurin, they are suitable as test organisms. Our work has confirmed this. Our experiments were designed to determine if a sponge or holothurian is toxic to fish and to obtain a rough approximation of the degree of toxicity (*6*). Marine fish were used in many of the studies to ensure that local fish species are responding to toxins from sponges and holothurians with which they are associated. Toxicity in holothurians was tested separately for the body wall, viscera, and Cuvierian tubules. The data presented here are based on extracts from the body wall since this seems to be the most impor-

Table 1. Toxicity of holothurians at various latitudes. For the techniques used by Bakus see (*3*) and (*6*). Bakus defines holothurians as highly toxic if the fish dies within 15 minutes (usually less than 10 minutes) and mildly toxic if the fish dies in 20 to 45 minutes. Yamanouchi [see (*5*) for techniques] used highly toxic if the mean survival time of ten fish (together) was up to 60 minutes and mildly toxic if it was 116 to 173 minutes. Abbreviations: HT, highly toxic; MT, mildly toxic; GJB, Gerald J. Bakus; TY, T. Yamanouchi.

Locality	Latitude (°N)	Depth (m)	Number of holothurian species				Investigator
			Tested	Toxic	HT	MT	
San Juan Islands, Washington	48	0–100	12	3 (25%)	0	3	GJB
Onagawa, Japan	38	?	5	4 (80%)	1	3	TY
Seto, Japan	35	?	9	7 (78%)	5	2	TY
Santa Catalina Island, California	33	0–10	2	1 (50%)	0	1	GJB
Guaymas, Mexico	28	0–5	6	5 (83%)	5	0	GJB
Eniwetok, Marshall Islands	12	0–3	4	4 (100%)	4	0	GJB
Palau Islands, Pacific Ocean	7	0–10	11	11 (100%)	9	2	TY
Cocos Island, eastern Pacific	6	0–110	7	6 (86%)	6	0	GJB

Table 2. Toxicity of sponges at various latitudes. The investigator was Green; see (*6*) and (*10*) for techniques. Green defines sponges as highly toxic (HT) if the fish dies within 60 minutes, moderately toxic (Mod.T) if it dies in 61 to 120 minutes, mildly toxic (MT) if it dies in 121 to 720 minutes, and very mildly toxic (VMT) if it dies in 720 to 960 minutes.

Locality	Latitude (°N)	Depth (m)	Number of sponge species					
			Tested	Toxic	HT	Mod. T	MT	VMT
San Juan Islands, Washington	48	0–50	34	3 (9%)	3	0	0	0
Santa Catalina Island, California	33	0–40	44	9 (21%)	5	4	0	0
La Blanquilla Reef, Veracruz, Mexico	19	1–20	36	27 (75%)	12	2	5	8
Zihuatanejo Bay, Guerrero, Mexico	17	1–15	11	7 (64%)	1	1	0	5

tant protective structure for most holothurians. Tropical holothurians rarely eviscerate (*7*) and very few species that were studied have Cuvierian tubules for defense. Data are fewer for holothurians than for sponges partly because sponges are more diverse at the study sites.

In Table 1 our results for holothurians are compared with those of Yamanouchi (*5*). Only 25 percent of the holothurians studied in the state of Washington are toxic to fish, and this toxicity is mild relative to that of toxic tropical holothurians. The incidence of toxicity increases toward the tropics and reaches a peak (100 percent of the species studied in the Palau Islands) where coral reefs and associated fish exhibit a high degree of environmental heterogeneity and species diversity, respectively. The relatively high incidence of toxicity in holothurians of Japan may be related in part to the fact that the north equatorial current carries warm water to the southern half of Japan. Table 2 shows the same trend in toxicity, in that only 9 percent of the Washington sponges studied are toxic to fish, whereas 75 percent of the sponges studied on a coral cay near Veracruz, Mexico, are toxic. The three species of sponges that are toxic in Washington are widely distributed. Of 27 species of toxic sponges in Veracruz, 21 live exposed to potential fish predators and the remaining 6 live both exposed and unexposed to fish (that is, under corals). Of an additional nine species of sponges that were found to be nontoxic to fish, six live unexposed to fish, one is both unexposed and exposed, and two are exposed (one of the two produces a purple exudate that is noxious to fishes). If data were available from well-developed reefs of the equatorial west Pacific, it would be expected that nearly all the sponges growing exposed to fish predators and grazers would be toxic or chemically noxious to them, or both. This conclusion is based on the trends shown here and on evidence from experiments in the Virgin Islands and at Eniwetok Island (*2*).

Underwater observations suggest that fish only mildly threaten sponges and holothurians in Washington. On 14 dives to depths of 15 to 20 m at San Juan Island during July and August 1972, we did not observe more than six species of predacious fish living on the sea floor at any site, and we seldom observed any predacious fish in the water column, even when underwater visibility reached 10 m. Moreover, fish that were observed displayed no interest when we attempted to hand-feed them with crabs, the viscera of sea urchins, or toxic sponges or holothurians. We can only assume that they were satiated at these times. It has been shown that asteroids become the major predators of many benthic invertebrates on hard bottoms in cold temperate and higher latitudes (*8*). In strong contrast, there is no marine habitat that supports such a high diversity and standing crop of fish as that of coral reefs, and it is well known that fish predation and grazing on benthic invertebrates is often very intense there, particularly in the equatorial Pacific (*2, 3, 9*). Our field and laboratory experiments with holothurians cut into bite-sized pieces show that starved fish rarely consume the toxic tropical forms, but readily eat mildly toxic nontropical species (*2, 3, 7, 9*). Similarly, our experiments indicate that all fish studied reject highly toxic sponges and are killed by the sponge if force-fed with bite-sized pieces (*2, 10*). A few species of tropical fish consume toxic sponges in nature (*10, 11*).

It could be argued that toxicity in holothurians is due to the physical effect of high temperature on the biochemical production of steroid saponins, because holothurin has been found in considerably greater concentrations in holothurians during warmer than colder seasons in Japan (*12*). This would tend to support the idea that holothurin is a by-product of metabolism, increasing directly with temperature. However, certain tropical holothurians (*3*) and many cryptic tropical sponges are nontoxic to fish (*1, 10*). Very little is known about the chemistry of sponge toxins or the effects of temperature on these toxins.

We recognize that there are many kinds and causes of toxicity in marine organisms. The biological impact of marine fish on benthic invertebrates and of introduced fish is well documented (*1–3, 7, 9, 13*). We believe that fish predators and grazers must play a very important selective role, leading to the production and maintenance of chemical defense mechanisms (that is, allomones) in certain benthic invertebrates of tropical waters, especially those associated with coral reefs. We assume for the present that noxious and toxic chemical defenses in these organisms are often by-products of metabolism, which have been altered and concentrated by natural selection over various periods of time. Although certain of these toxins may have arisen long ago, we believe that natural selection is continuing to select for noxious chemical products and that this process may in some cases give rise to the production of toxins; both of these provide an effective defense mechanism against many predacious fish.

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The homogenized sponge is centrifuged and the supernatant is evaporated. The crude extract is dissloved in 300 ml of fresh water in a finger bowl in which a goldfish is placed, and the behavior of the fish is timed and recorded. Control experiments are done simultaneously.

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Isolated Brain Microvessels: A Purified, Metabolically Active Preparation from Bovine Cerebral Cortex

Abstract. *A purified, metabolically active preparation of brain microvessels was isolated from bovine cerebral cortex by using a simple procedure involving mild disruption of the tissue by homogenization and trapping of the vessels on nylon sieves. This preparation permits in vitro metabolic and structural studies of small blood vessels.*

Knowledge of the metabolism and structural composition of isolated blood vessels is important in studies of the biochemistry and pharmacology of this organ system, which is a primary site for a wide range of pathologic disturbances, including arteriosclerosis and diabetes mellitus. The microvessels, defined as those with diameters less than 300 μm, are frequently affected by such diseases to as great an extent as larger vessels like the aorta (*1*). However, only the latter have been the subject of extensive biochemical investigations (*2*). The difficulty of readily obtaining sufficient quantities of microvessels for experimentation is one reason for this disparity. The development in our laboratory of simple isolation procedures involving mild homogenization and sieving for obtaining morphologically intact and metabolically active preparations of kidney glomeruli and retinal blood vessels suggested that similar techniques might be useful for the isolation of microvessels from other tissues (*3*). The successful application of these techniques to the isolation of microvessels from cerebral cortex provides a readily obtained preparation of small blood vessels suitable for metabolic investigations of the microcirculatory system in general as well as those aspects peculiar to vessels of the central nervous system.

Figure 1 shows a typical preparation of brain microvessels at low (Fig. 1a) and higher (Fig. 1b) magnification. Gray matter is obtained from the cerebral cortex of bovine brain. For optimum preservation of metabolic activity, the vessels should be isolated promptly from brains removed from the animals immediately after slaughter, and the isolated vessels should be transported to the laboratory in cold oxygenated buffer to minimize the effects of anoxia on the tissue. Pieces of cortical tissue are homogenized in Earle's balanced salt solution buffered with HEPES (*4*) (1 : 1 by volume) with ten vertical strokes of a hand-held loosely fitting Teflon pestle in a smooth glass tube (*3*). The homogenate is poured over a 153-μm nylon sieve (*5*), and the material remaining on the sieve after washing with buffer is rehomogenized, resieved, and washed as before. A highly enriched preparation of microvessels with the appearance of fine threadlike strands is caught by the sieve, while the bulk of the nonvascular tissue of a fine granular nature is not retained. The vessels are freed of any adhering or accompanying nonvascular contamination either by homogenizing them with two or three additional strokes, resieving, and rewashing, or by "combing" through a suspension of the preparation in a petri dish with a piece of 210-μm nylon mesh. The vessel strands

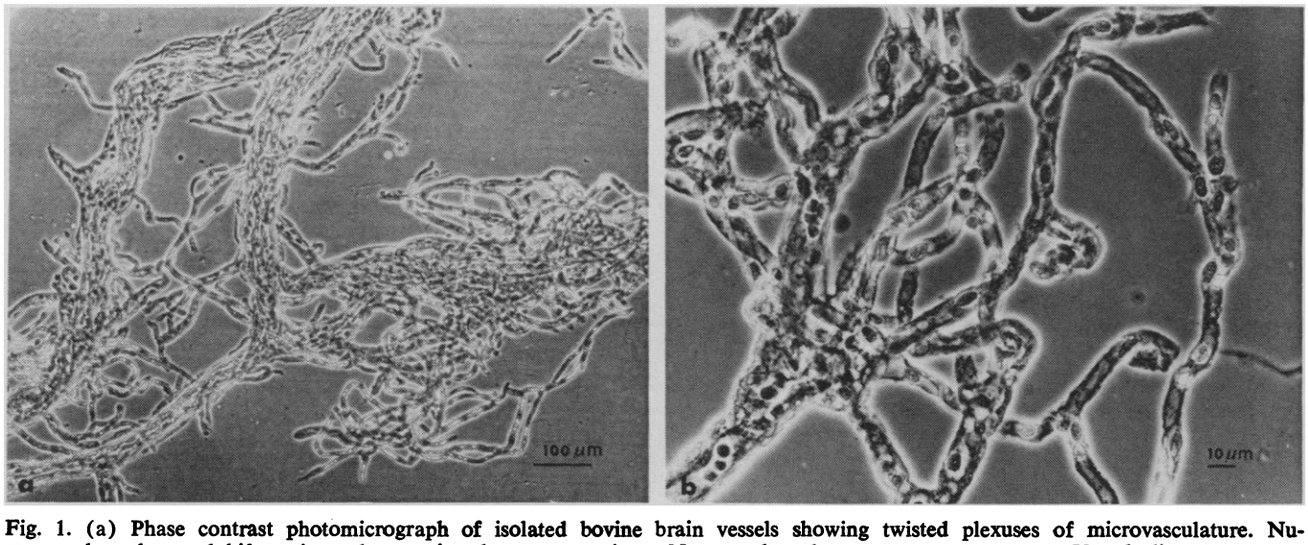

Fig. 1. (a) Phase contrast photomicrograph of isolated bovine brain vessels showing twisted plexuses of microvasculature. Numerous branches and bifurcations characterize these preparations. Nonvascular elements are not present. Vessel diameters range from 6 to 80 μm (× 85). (b) Phase contrast photomircograph at higher magnification showing vascular arborization in isolated bovine brain vessels. Clusters of red blood cells occupy vessel lumina. Endothelial cell nuclei are particularly evident at this magnification (× 350).