costae assayed by thin-layer chromotography. If lipids were a component of the costa, they may have been removed by the hemolysin or the phospholipase treatment, or both, and must not have a role in structural integrity.

The GLC analysis of purified costae showed the carbohydrate component to be 95 percent glucose, 1.4 percent glucosamine, 0.6 percent ribose, 0.4 percent mannose, and 2.6 percent an unidentified moiety. Glucose is the main component, and whether the sugars found in small amounts are part of the costa or are derived from contaminating material is conjectural.

Protein (about 5 percent) is a vital component of the structure because exposure of purified costae to trypsin resulted in disintegration and apparent solubilization of the entire structure. Trypsin has been reported to destroy costae in trichomonads (*7*), which implied a protein component of costae. Also, it has been hypothesized that costae are composed of collagen-like material because of their striated appearance (*8*), even though collagenase did not affect costal integrity (*7*).

Tritrichomonas foetus contains substantial quantities of glycogen (10 to 30 percent) depending on growth conditions, and rapid turnover of the cellular glycogen has been shown to occur (*9*). Because of the high glucose content of purified costae, the organelle may have an energy-generating function or may serve as an energy reserve, in addition to the structural function served by its rigidity. The structural and other functions of costae require reconsideration in light of our findings of the chemical composition of this organelle in the purified state.

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Maculotoxin: A Neurotoxin from the Venom Glands of the Octopus *Hapalochlaena maculosa* Identified as Tetrodotoxin

Abstract. *Maculotoxin, a potent neurotoxin isolated from the posterior salivary glands of the blue-ringed octopus,* Hapalochlaena maculosa, *has now been identified as tetrodotoxin. This is the first reported case in which tetrodotoxin has been found to occur in a venom.*

The blue-ringed octopus, *Hapalochlaena maculosa*, is a small octopus that is common along the coast of Australia. It derives its popular name from its ability to display brilliant blue rings of color on its skin when disturbed. After a number of human fatalities (*1, 2*) attributed to the bite of this octopus, the chemistry (*3, 4*) and pharmacology (*5*) of extracts of its posterior salivary glands were investigated. The pharmacological action of maculotoxin, the principal neurotoxin present in extracts of these venom glands, was described (*6*) as being similar to that of tetrodotoxin, although some differences between them have been noted (*7*). We now report the isolation of pure maculotoxin in quantities sufficient to yield information concerning its chemical nature. Direct spectral and chromatographic comparison of maculotoxin with tetrodotoxin shows them to be indistinguishable. *Hapalochlaena maculosa* is the first species in which tetrodotoxin has been found in extracts of the venom glands, in contrast to all other known cases in which it occurs as a poison in the skin, muscle, liver, ovaries, or eggs (*8, 9*).

Specimens (250) of *H. maculosa* were collected in early autumn off the South Australian coast. Upon collection the octopuses were frozen. The posterior salivary glands were removed by dissection and homogenized with 300 ml of 3 percent acetic acid. The homogenate was centrifuged at 16,000*g* for 0.5 hour, and the supernatant was decanted, frozen, and dried. The residue was extracted four times with 3 percent acetic acid. The combined material extracted by aqueous acetic acid weighed 13.4 g and had an activity of 2 mouse units (*8*) per milligram (M.U./mg). The extract was dissolved in 500 ml of 3 percent acetic acid and concentrated to 100 ml by passage through an Amicon Diaflo UM2 ultrafilter (retention > 1000 daltons). The concentrate was filtered by dialysis at a constant volume with 1 liter of acetic acid. This yielded 6.3 g of filtrate having an activity of 3 M.U./mg; 7.0 g of inactive material was retained by the filter.

The filtrate was dissolved in 15 ml of 0.1*M* ammonium acetate at *p*H 6.0 and applied to a column (2.5 by 40 cm) of CM-Sephadex C-25 gel (ammonium form). The column was eluted with a linear ionic strength gradient of 0.1 to 0.4*M* ammonium acetate at *p*H 6.0. The eluted lethal fractions were combined and freeze-dried; they afforded 179 mg of material having an activity of 70 M.U./mg. Only one group of lethal fractions was observed even after elution of the column with five void volumes of 3 percent acetic acid. Savage and Howden (*4*), however, appeared to have detected two toxins when the isolation was carried out by a different method. The lethal material (179 mg) obtained from the initial ion-exchange chromatogram was rechromatographed three times on a CM-Sephadex C-25 column under the same conditions as before. This afforded 1.8 mg of pure maculotoxin having an activity of approximately 7000 M.U./mg. Tetrodotoxin from Sigma Chemical Company had comparable toxicity.

A 30-mg sample of the diafiltrate was subjected to isoelectric focusing, at a *p*H gradient of 7 to 11 with LKB ampholines in a support gel of superfine Sephadex G-75 on an LKB Multiphor instrument. An initial current of 20 ma at 400 volts was applied for 20 hours; the voltage was then increased to 1000 volts for 8 hours. Only one band having an isoelectric point of 8.5 ± 0.1 contained lethal material.

The purification of maculotoxin was also monitored by thin-layer chromatography on Merck silica gel GF 254 plates, which were developed with an isopropanol, acetic acid and water system (14:1:5). Maculotoxin was detected as a light yellowish-brown spot by spraying the plate with a mixture of vanillin and sulfuric acid, and then heating to 110°C, or as a yellow spot under long-wave-

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length ultraviolet light by spraying with 10 percent potassium hydroxide in methanol and heating at 110°C. The maculotoxin fractions obtained after three ion-exchange chromatograms gave spots at R_F 0.31 and 0.12 with potassium hydroxide in methanol. Elution from a non-visualized plate with 3 percent acetic acid showed that the only lethal compound was located between R_F 0.3 and 0.4. A final CM-Sephadex C-25 column chromatogram afforded 1.8 mg of pure maculotoxin (0.006 percent, by weight, from posterior salivary glands), which showed only one spot, alone or mixed with authentic tetrodotoxin (Sigma), on thin-layer chromatography.

Pure maculotoxin was twice freeze-dried with D_2O then dissolved in 20 μl of 3 percent completely deuterated acetic acid (CD_3CO_2D) in D_2O. Its proton nuclear magnetic resonance spectrum (JEOL; 100 Mhz) showed a singlet at 2.72 (CD_2HCO_2D), a doublet centered on 2.98 (J = 9.5 hertz), a multiplet with peaks at 4.62 and 4.88, a large proton peak at 5.39 (HOD), and a doublet centered on 6.14 parts per million (ppm) (J = 9.5 hertz). This spectrum was identical with that of authentic tetrodotoxin examined under the same conditions. The pair of doublets at 2.98 and 6.14 ppm, which are the hallmarks of tetrodotoxin (*8*), were shown to be coupled by double irradiation.

Our identification of tetrodotoxin as the principal neurotoxin in the venom glands of *H. maculosa* lengthens the list (*8*) of diverse creatures in which this toxin occurs. The extraordinary ubiquity of tetrodotoxin makes it unique in this respect among animal neurotoxins. The role played by tetrodotoxin in *H. maculosa* is perhaps more obvious than in other species, since *H. maculosa* uses its venom to immobilize or kill its prey of small crayfish and crabs (*1*).

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Axial Differences in the Musculature of Uropeltid Snakes: The Freight-Train Approach to Burrowing

Abstract. *The shield-tailed snakes (family Uropeltidae) extend and widen the tunnels in which they live by alternately curving and straightening the anterior portion of their vertebral columns within the skin, a burrowing method that proves to be most effective for tunneling amid roots and rocks, as well as for producing tunnels wider than the trunk through unpredictably heterogeneous substrates. The muscles of the anterior portion of the uropeltid trunk are larger and thicker than those of the posterior and are further modified by the inclusion of large amounts of myoglobin, numerous mitochondria, and diverse other ultrastructural and enzymatic specializations, which presumably represent adaptations for sustained work loads. The very much thinner, serially homologous, but unmodified musculature of the posterior trunk occupies only a much smaller fraction of the cross-sectional area. This regional modification increases the effectiveness of the posterior body for storing viscera and developing embryos.*

Theoretical analysis has suggested that reptiles living underground reflect the conflict between the advantage of traversing a relatively narrow tunnel (the construction of which requires minimal energy per unit length) and the internal restructuring forced by the need to reduce the diameter of various components, such as the feeding mechanism, the ear, and the reproductive system (*1*). This conflict is particularly important when the animal is harvesting small, ran-

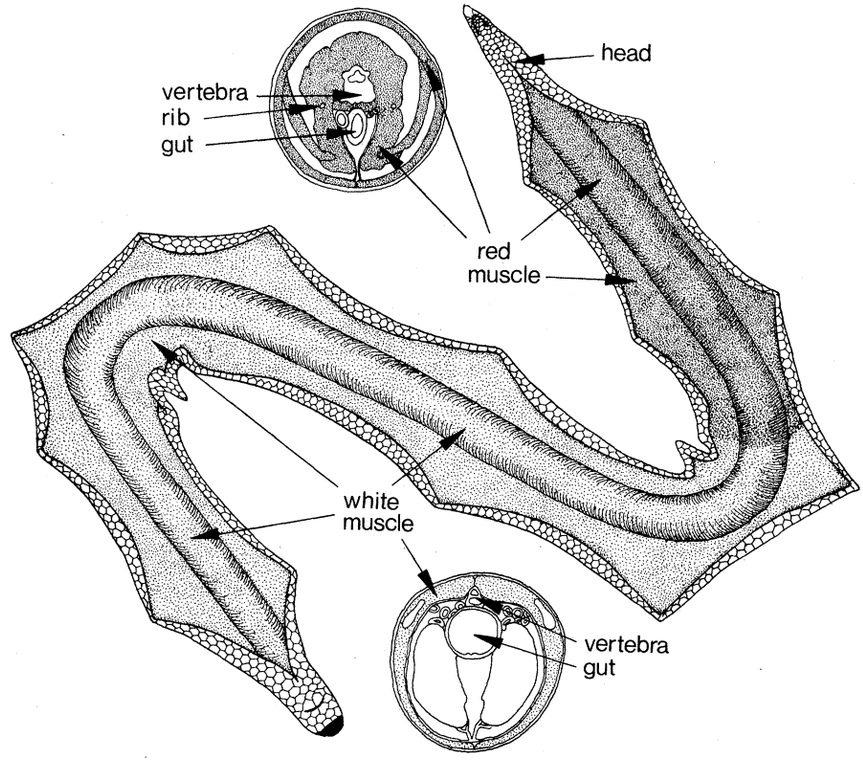

Fig. 1. Sketch of a partly skinned speciment of *Rhinophis drummondhayi* showing the extent of red and white zones of axial musculature. The insets show that the anterior (red) musculature occupies a much larger fraction of the trunk's cross section than does the posterior (white muscle).

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