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 nonvisualized 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-

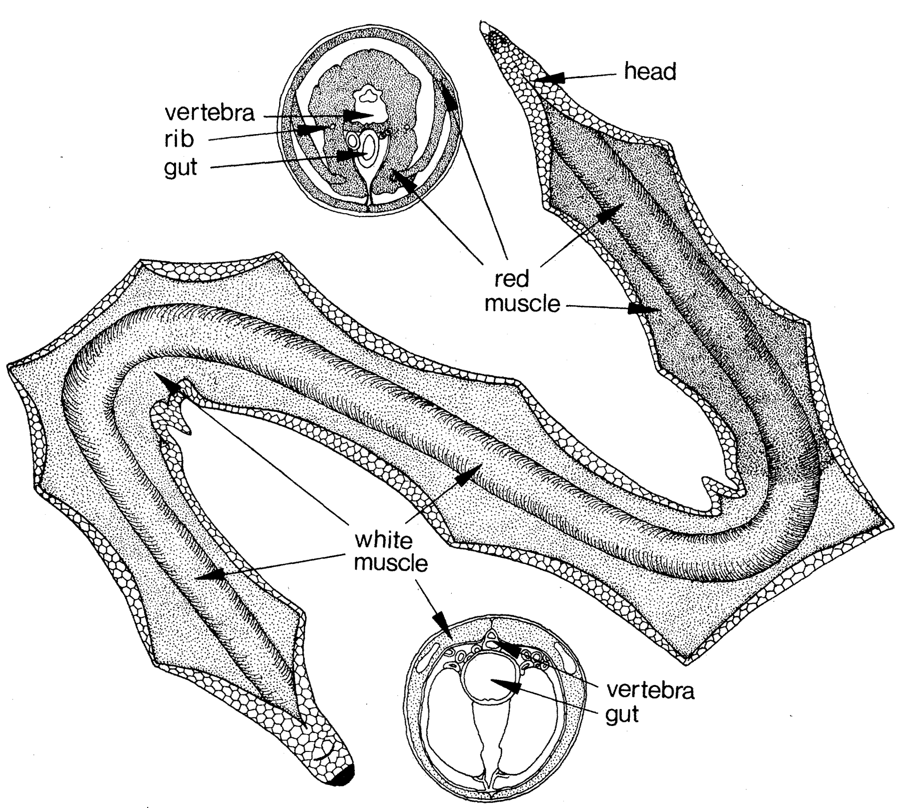

Fig. 1. Sketch of a partly skinned speciment of *Rhinophis drummondhayi* showing the extent of red and white zones of axial musculature. The insets show that the anterior (red) musculature occupies a much larger fraction of the trunk's cross section than does the posterior (white muscle).

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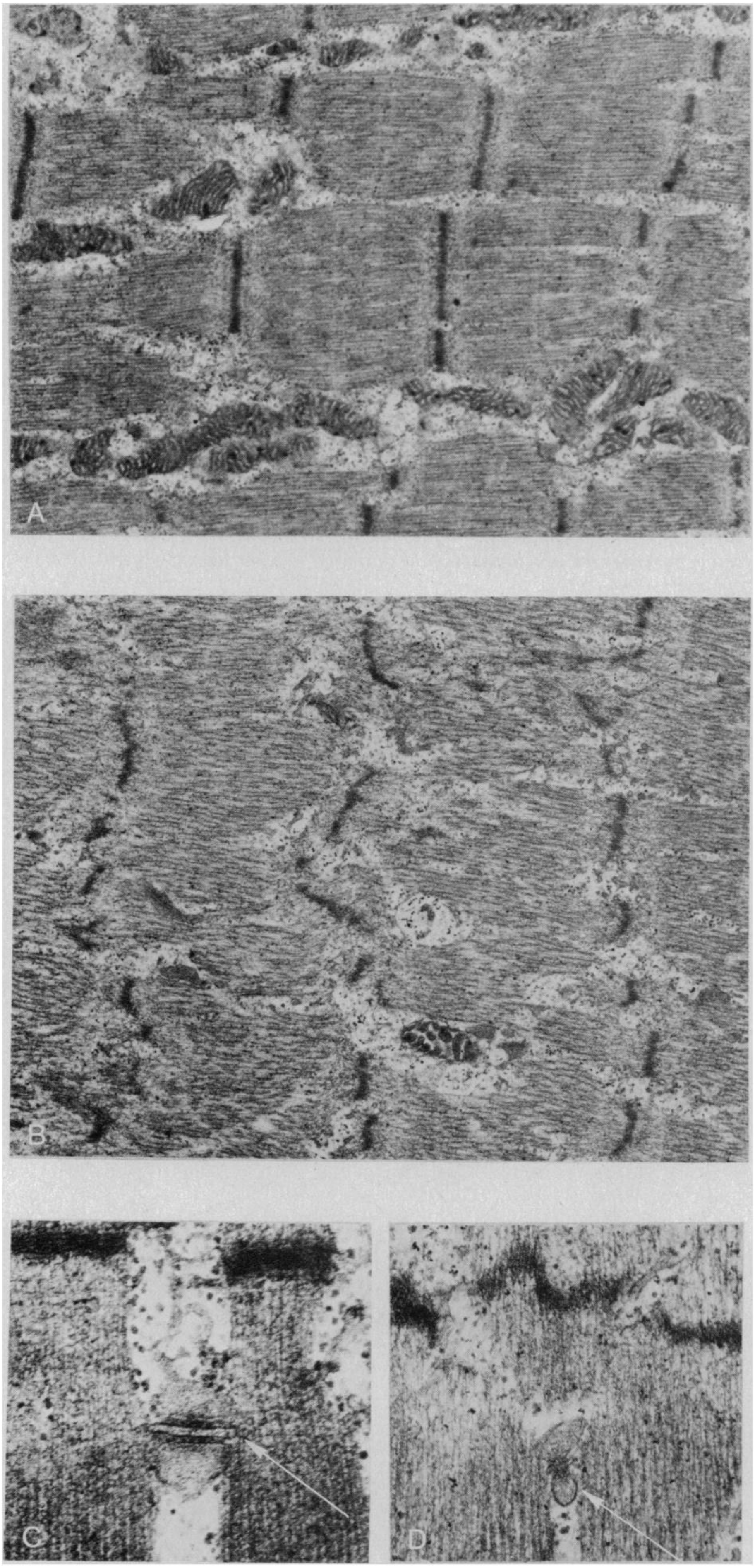

domly distributed food resources (such as worms or insect larvae), which require search down old and new tunnels (*2*). Even then the energetic pattern is not the only critical influence since the body diameter will limit the size of organs. The dimensional constraint is particularly critical for those organs that, unlike lungs and liver, must maintain a shape-dependent function, as does the oviduct containing the serially arranged developing young.

The advanced members of the endemic family of shield-tailed snakes (Uropeltidae) of India and Sri Lanka (*3*) appear to have resolved this problem in a unique fashion that (i) increases the relative area of the usable space (actually of the coelom) within the overall cross-sectional area of the trunk and (ii) produces a tunnel wider than the trunk. Those axial muscles of the uropeltids joining the vertebrae and the paired ribs, as well as the muscles connecting this mass to the integument, appear to be drastically thickened in the first fifth of the vertebral column (or to just posterior to the heart). Not only are the vertebral and costal skeleton and muscles much heavier (occupying more than 60 percent of the cross section of the body near vertebra 15, compared to less than 25 percent near vertebra 120, so that the coelom occupies about 6 percent and 50 percent, respectively) (Fig. 1), but the fibers are otherwise modified as well (Fig. 2). The modification is most obvious in fresh specimens in which the anterior muscle mass is opaque and deep red, in contrast to the almost translucent-to-clear-to-whitish color of the serially homologous posterior muscles. Both hypertrophy and red color are seen at birth.

Regional differentiation of body musculature also is evident at the cytological and molecular levels. The electron micrograph (Fig. 2A) shows that this anterior musculature has fibrils that are separated by regular clefts containing numerous mitochondria. The mitochondria are large and elongated and have dense cristae. The triads of the transverse tubular system are located at the level of the junction of A and I bands (Fig. 2C, arrow). The neighboring Z lines are arranged in regular, straight lines. These muscles contain fat droplets. The poste-

Fig. 2. Electron micrographs of costocutaneous muscles of *Rhinophis drummondhayi* from Namunukula, Sri Lanka. (A) Red muscle, ×4,600; (B) white muscle, ×4,600; (C) triad structure in the red muscle, ×56,000; (D) triad structure in the white muscle, ×56,000.

rior muscle (Fig. 2B) has fibrils interspersed with less regular clefts and has only few mitochondria. The mitochondria are small and short and have very dense and thick cristae. The triads (Fig. 2D, arrow) are rare, though the sarcoplasmic reticulum is well developed. The Z lines frequently have an irregular zigzag appearance. No lipid droplets were seen in the white muscle.

The color difference is due to the presence of approximately 2 g of myoglobin per 100 g in red muscle and its almost complete absence from white muscle. Red muscle also exhibits higher activities of malate and isocitrate dehydrogenases and of both glutamic-oxaloacetic and glutamic-pyruvic transaminases. Glycolytic enzymes do not show regional differentiation (Table 1). The cytological and biochemical characteristics of the anterior musculature are similar to those of mammalian muscle adapted for endurance exercise (*4*). Apparently, the axial and integumentary muscles contain mainly oxidative "red" muscles in the anterior zone with a serial transition posteriorly to an arrangement of glycolytic "white" fibers.

The arrangement of the red muscle is directly associated with the peculiar method of tunnel formation shown by uropeltids (Fig. 3). When extending a tunnel system by forming new branches, these snakes form the anterior vertebral column into a sequence of hairpin turns pressing the external loops against the tunnel wall (*5*). The zone in firm contact with the tunnel walls (Fig. 3) absorbs the reaction forces imposed when driving the tip of the uniquely pointed head into the substratum. As soon as the cephalic cone has penetrated to about the level of the anterior vertebrae (at which level the trunk has achieved full diameter) the straightened neck is again thrown into a new set of curves. The lateral extent of these not only provides a transmission base for the reaction forces of the next push but in itself tends to widen the tunnel beyond the diameter of the neck.

The exertion of lateral forces on to the tunnel wall is enhanced by the loose connection between the axial mass and the integument. The multiple curves in the vertebral column are formed within the loose envelope of the anterior skin which widens but does not itself curve (*6*). This loose connection between axial mass and integumentary envelope allows a smooth widening of the anterior body and achieves a more regular widening of the tunnel.

The concentration of the machinery for widening the initial divot formed by the penetrating head has several advantages. (i) As the propulsive machinery is concentrated in the anterior portion of the trunk, only a minimal fraction of the cross-sectional area of the posterior trunk must be allocated for support and propulsion; the remainder is reserved for storage. (ii) The tunnel formed is considerably wider than the widest part of the trunk. This limits the risk of exerting pressure on the sides of the body and possibly damaging entrained tissues when the animal is temporarily swollen because of a large meal or when it contains a series of embryos near term (*7*). (iii) The burrowing method provides an ideal tunneling device for an unpredictably inhomogeneous substratum. The initial divot driven by the head is quite narrow and will be deflected by roots or

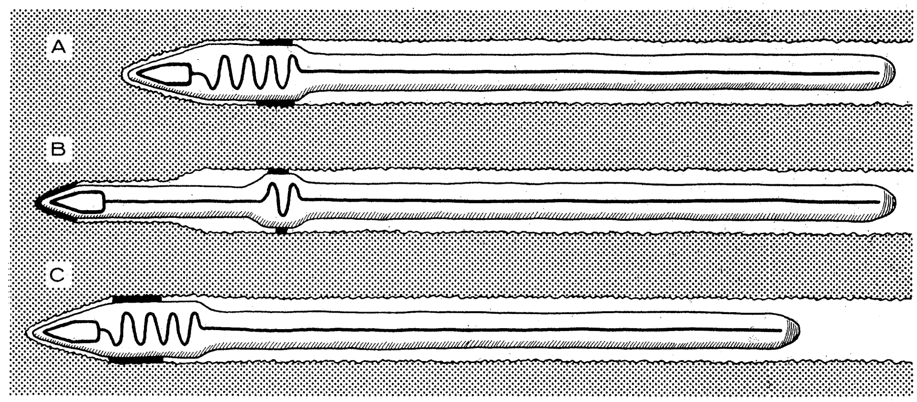

Fig. 3. Tunnel penetration pattern of an advanced uropeltid. (A) The snake has curved its anterior vertebral column within the integumentary envelope and stems its sides against the walls, thus widening the tunnel diameter and providing a base from which to penetrate farther. The head is next forced into the soil forming a tunnel extension of narrow diameter (B) that is widened (C) as the posterior portions of the vertebral column are pulled anteriorly and curved against the tunnel wall in order to form a base for further penetration. Dark areas between the snake and the tunnel wall indicate the zone of the snake in firm contact with the wall.

Table 1. Comparison of proteins from red (anterior) and white (posterior) muscle. Concentrations of myoglobin were estimated spectrophotometrically for *Rhinophis phillipinus*; absorption maxima of oxymyoglobin are 581 and 544 nm; its oxyhemoglobin has maxima at 578 and 541 nm. Relative activities of the enzymes were estimated from rates of staining on starch gel electropherograms: The designation +++ indicates high activity; ++, moderate activity; +, weak activity; −, activity not detected.

Proteins	Red muscle	White muscle
Concentration		
Myoglobin	1.81 ± 0.52 g/100 g*	< 0.01 g/100 g
Relative activity		
Dehydrogenases		
Glycerol-3-phosphate (E.C. 1.1.1.8)	++	+
Lactate-1† (E.C. 1.1.1.27)	+	+
Lactate-2† (E.C. 1.1.1.27)	+++	+++
Lactate-3† (E.C. 1.1.1.27)	+	−
Malate (E.C. 1.1.1.37)	+++	++
Malate (E.C. 1.1.1.40)	+	+
Isocitrate (E.C. 1.1.1.42)	+++	++
Phosphogluconate (E.C. 1.1.1.44)	+	+
Transferases		
Glutamate-oxaloacelate-1† (E.C. 2.6.1.1)	+++	+
Glutamate-oxaloacelate-2† (E.C. 2.6.1.1)	++	+
Glutamic-pyruvic (E.C. 2.6.1.2)	++	+
Adenylate kinase (E.C. 2.7.4.3)	++	++
Creatine kinase (E.C. 2.7.3.2)	++	++
Phosphoglucomutase (E.C. 2.7.5.1)	++	++
Glucosephosphate isomerase (E.C. 5.3.1.9)	+	+
Hydrolases		
Leucyl-proline dipeptidase (E.C. 3.4.13.9)	+	+
Valyl-leucine dipeptidase-1† (E.C. 3.4.11)	++	−
Valyl-leucine dipeptidase-2† (E.C. 3.4.11)	++	++
Leucyl-glycyl-glycine peptidase (E.C. 3.4.11)	++	++
Umbelliferyl-acetate esterase (E.C. 3.1.1.1)	++	++
Adenosine deaminase (E.C. 3.5.4.4)	+	+

*Mean of 20 specimens ± standard deviation; range, 0.89 to 2.91. †Loci are numbered in order of decreasing anodal mobilities of their products.

rocks. When it passes close to such effectively nondeformable and nondisplacable objects, the opposite wall of the tunnel will be compressed unevenly so that the final tunnel achieves its full if meandering diameter by extra asymmetric compression of the softer zones.

Whereas the posterior body musculature is capable of throwing the trunk into undulations, analysis of films shows clearly that (i) relatively little continuous force is exerted by this zone and (ii) this posterior half of the body is relatively inactive in propulsion. Furthermore, the shield-tailed snakes do not use their caudal tip to stem (that is, to absorb) the reaction forces obtained as their head starts to enter and extend crevices; thus they differ from worm snakes, families Typhlopidae and Leptotyphlopidae. The posterior vertebrae are relatively shallower than the anterior, which indicates that this portion of the trunk is not equipped to absorb major or continuous forces.

Consequently, the uropeltid body may be conceived of as analogous to a freight train. All its propulsive machinery is concentrated in the anterior portion, while the posterior trunk serves mainly for the indirectly powered transport of the viscera. In the same way that the caboose provides terminal protection for a string of freight cars, the uropeltid's modified caudal shield protects the snake's distal end (*8*). Movement along the tunnel is unidirectional; thus uropeltids cannot burrow backward and have trouble backing up unless the tunnel is smooth and well formed.

The adaptive pattern here shown is not only interesting as an ecological response to a particular set of environmental conditions but also documents the fact that the adaptive response of muscle is not restricted to mere hypertrophy or reduction. In this case, it involves a general modification of the enzyme system and fibrillar arrangement within regional groupings of muscles, though it is probable that the evolutionary change actually involved a simultaneous loss of oxidative capacity in the posterior portion of the trunk and its enhancement in the anterior.

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2. The energy that a compression burrower has to expend in generating a unit length of tunnel will increase at least with the first power of the tunnel's diameter. At the same time the burrower's mass will determine its metabolic rate and with this its absolute energy requirement. Other things (prey capture and locomotor techniques) remaining equal, a reduction of the relative diameter of the head forces a decrease in the size of prey objects that may be killed and ingested, and with this increases the number of prey-capture operations required per unit time.
3. The muscular and functional differentiation here described has been analyzed in detail in the Sri Lankan uropeltid species *Rhinophis blythi*, *R. drummondhayi*, *R. dorsimaculata*, *R.* sp., *R. philippinus*, *R. trevelyanus*, *Uropeltis melanogaster*, and *U. phillipsi*, and the Indian species *U. liura*. The differentiation is probably absent in the Indian genera *Brachiophidium*, *Melanophidium*, *Plecturus*, and *Platyplecturus*; it is definitely absent in the Indian *Teretrurus rhodogaster* and in the Sri Lankan genus *Pseudotyphlops*, the largest of the uropeltids. Although *Pseudotyphlops* represents a highly advanced set of character states, in such aspects as the nature of its caudal shield, it is apparently unable to tunnel in the low-country soils when these are dry and hard. It then remains in its deeper tunnels and only leaves or extends them when rain softens them, dropping penetrometer readings from 4.5 to 0.5 k/cm^2.
4. Muscles of mammals adapted to vigorous, prolonged exercise (such as long-distance running) have increased work capacity and ability to oxidize pyruvate and fatty acids. These capabilities are reflected in increased mitochondria and activities of a number of citric cycle enzymes, transaminases, and mitochondrial proteins [B. Parnow and B. Saltin, Eds., *Adv. Exp. Med. Biol.* **11** (entire issue) (1971); P. A. Mole, K. M. Baldwin, R. L. Terjung, J. O. Holloszy, *Am. J. Physiol.* **224**, 50 (1973)].
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6. This phenomenon accounts for several sets of misapprehensions in the taxonomic literature. One of these is the argument that uropeltids are generally characterized by carrying the head bent to one side ["Very frequently the longitudinal axis of the head is not the same as that of the neck, the head being impressed on one side, as if it had been dislocated during some effort of the snake to penetrate the soil"—A. C. L. G. Günther, *The Reptiles of British India* (Ray Society, London, 1864), p. 182]; this is the condition of a snake preserved with the axial musculature hypercontracted, thus forcing the head to one side or the other (unless restrained in a straight position by the tunnel wall). Another such artifact produces the descriptions "forebody swolled and knuckled" [F. Wall, *Ophidia Taprobanica or the Snakes of Ceylon* (H. R. Cottle, Colombo, Ceylon, 1921), p. 30] and "pronounced thickening of the nuchal area" [P. E. P. Deraniyagala, *A Colored Atlas of Some Vertebrates from Ceylon*, vol. 3, *Serpentoid Reptilia* (Government Press, Colombo, Ceylon, 1955), p. 8]; such descriptions are applicable to specimens that have the axial musculature of the neck slightly contracted so that the neck is curved and the integument appears widened.
7. This function probably provides one explanation for why the neck curvature method of tunnel widening is found in uropeltids and most caecilians but not in most members of the order Amphisbaenia except for *Agamodon compressum*. Most amphisbaenians bite pieces out of their prey rather than swallowing it entire as do uropeltids. The exceptional amphisbaenian *A. compressum* has a trunk of highly ribbon-shaped cross section and might presumably encounter some lateral limitation when harvesting seasonally abundant foods and when it is pregnant. The importance of pressure during pregnancy may be documented by the observation that in May 1976, at two localities, in a series of the uropeltid, *R. philippinus*, more than 20 percent of the individuals collected aborted fully formed but still immobile young that were estimated to be within 1 month of term. It is uncertain whether the abortion reflected only the trauma of being compressed while being dug up and shaken about, or also the general excitement.
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9. We thank the many friends and colleagues who facilitated the fieldwork, assisted in part by the Smithsonian Institution's Entomological project in Sri Lanka, the National Museum, Colombo, and aid from Professor R. J. Rajendran and Mr. P. Fernando. Drs. B. Carlson, J. A. Faulkner, G. Gorniak, and L. Maxwell commented on the manuscript. Preparative work involved the aid of Dr. D. F. Gartside and NSF grants BMS 71 01 380 (C.G.) and BMS 73 01 252 (H.C.D.). The drawings were prepared by R. Heberer.

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Intercellular Communication in Insect Development Is Hormonally Controlled

Abstract. *Cellular coupling in the insect epidermis changes in a characteristic way during metamorphosis. In vitro, β-ecdysone mimics the initial phase of these changes by increasing electrical coupling. Both adenosine 3′,5′-monophosphate (cyclic AMP) and* Ca^{2+} *reverse natural and β-ecdysone–stimulated changes, which suggests that ecdysone could work on communication through changes in cyclic AMP and* Ca^{2+} *levels. The transient changes in intercellular communication before metamorphosis may reflect the timing of the signals that trigger proliferation and the generation of new spatial patterns in the epidermis.*

Growth regulation in a developing tissue requires that the component cells communicate with each other. Over short ranges, the transmission of growth-regulating and morphogenetically important molecules through specialized membrane junctions has been proposed as a likely means of intercellular communication (*1*). The cytosol of normal nonexcitable cells is connected by permeable pathways that traverse the plasma membrane at gap junctions (*2*) that remain open throughout the cell cycle (*3*) and, in postembryonic tissues, allow the intercellular transfer of molecules with molecular weights less than 1000 (*4*). The pathway is an obvious candidate for the transmission of cell division initiators and the feedback regulation of growth in multicellular tissues (*1*).

In the insect, cell growth is periodic and under hormonal control (*5*). The in-