out excision repair. Mutagens that can both intercalate and form a covalent bond with DNA, such as the quinacrine–half mustard mutagen ICR 191, are much more mutagenic in reverting a particular histidine mutation in a strain without excision repair than in a strain with repair, while simple intercalating mutagens such as quinacrine or 9-aminoacridine are equally effective on the two strains (*11*). Benz[*a*]anthracene 5,6-oxide is negative, at the level of sensitivity of our test, in reverting the *hisC3076* mutation in a *uvrB*+ strain while, as shown in Table 1, it is quite effective on the double mutant TA1532 (that is, *hisC3076 uvrB*).

A number of other carcinogenic compounds have been shown to be powerful frameshift mutagens (*11*). A series of fluorene carcinogens are frameshift mutagens and one of the carcinogenic metabolic products with a reactive group, 2-nitrosofluorene, is one of the most potent frameshift mutagens we have ever tested (*16*). The well-known carcinogen 4-nitroquinoline *N*-oxide is also a frameshift mutagen in the *Salmonella* tests (*17*).

We think it is reasonable to propose that polycyclic hydrocarbons are carcinogenic because of the mutagenicity of epoxide intermediates formed during metabolism and that the mechanism of action may involve intercalation followed by covalent reaction. This proposed mode of action involving stabilization of DNA mispairing could explain the observations of Fahmy and Fahmy (*18*) that, in *Drosophila*, polycyclic hydrocarbons preferentially cause bobbed and minute mutations, which are large deletions in regions of duplicated gene clusters for ribosomal RNA and transfer RNA.

Note added in proof: We are indebted to J. R. Roth for raising the point that the epoxide induced mutations could conceivably be due to external frameshift suppressors, one class of which might be caused by base pair substitutions (*19*). We have ruled out this possibility by analyzing 48 epoxide induced revertants of TA1532, none of which were due to external suppressors.

BRUCE N. AMES
Department of Biochemistry,
University of California,
Berkeley 94720

P. SIMS, P. L. GROVER
Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
London SW3 6JB, England

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Radula Tooth Structure of the Gastropod Conus imperialis Elucidated by Scanning Electron Microscopy

Abstract. *Scanning electron microscopy of the hollow, harpoon-like radula tooth of the toxoglossan gastropod* Conus *has elucidated the structure and relationships of its component parts: apex, cutting edge, barbs, serration, adapical and basal openings of the lumen, external and internal folds of the shaft, and base. The functional roles of these components in prey capture are proposed.*

The radula of toxoglossan gastropods of the genus *Conus* is a simplified structure. It lacks the chitinous basal ribbon and associated musculature and supportive cartilage characteristic of this organ in most snails; it is quite short; and it has but two teeth in each row, only one of which is used at one time. However, these radula teeth are probably the most complex and highly modified in the Mollusca. Each tooth is adapted to perform at least three specialized roles almost simultaneously in capturing and overcoming prey: (i) the pointed apex must pierce the body wall of the prey; (ii) the tooth must catch and hold the body of the prey; and (iii) venom must be conveyed through the lumen of the tooth into the wound. In addition, in some species the base of the tooth must be held firmly by the tip of the proboscis during and after the injection process. The prey, typically polychaete annelids but other mollusks or fishes in some cases, is partially paralyzed by the venom and swallowed whole (*1*).

Although the general nature of the *Conus* radula tooth and something of the diversity of tooth form in the genus have been known for many years (*2, 3*), earlier studies have failed to elucidate the fundamental structural pattern of the tooth and the functional significance of its component features. This is due largely to the difficulty of determining the three-dimensional relationships of the internal and external parts of the enrolled, tubular, translucent, asymmetrical tooth by light microscopy (*2, 3*). By emphasizing surface details of teeth made opaque in preparation, and by af-

fording great depth of focus, the scanning electron microscope has made possible a more complete understanding of tooth structure and its functional significance (*4*).

We report here on our analysis of the structure of the radula tooth of *Conus imperialis* Linnaeus, chosen for initial study because of its comparatively large size and robust nature (Fig. 1), and because this type of tooth characterizes a small group of closely related *Conus* species with very specialized diets; the prey consists almost exclusively of polychaetes of the family Amphinomidae (fireworms) (*5*).

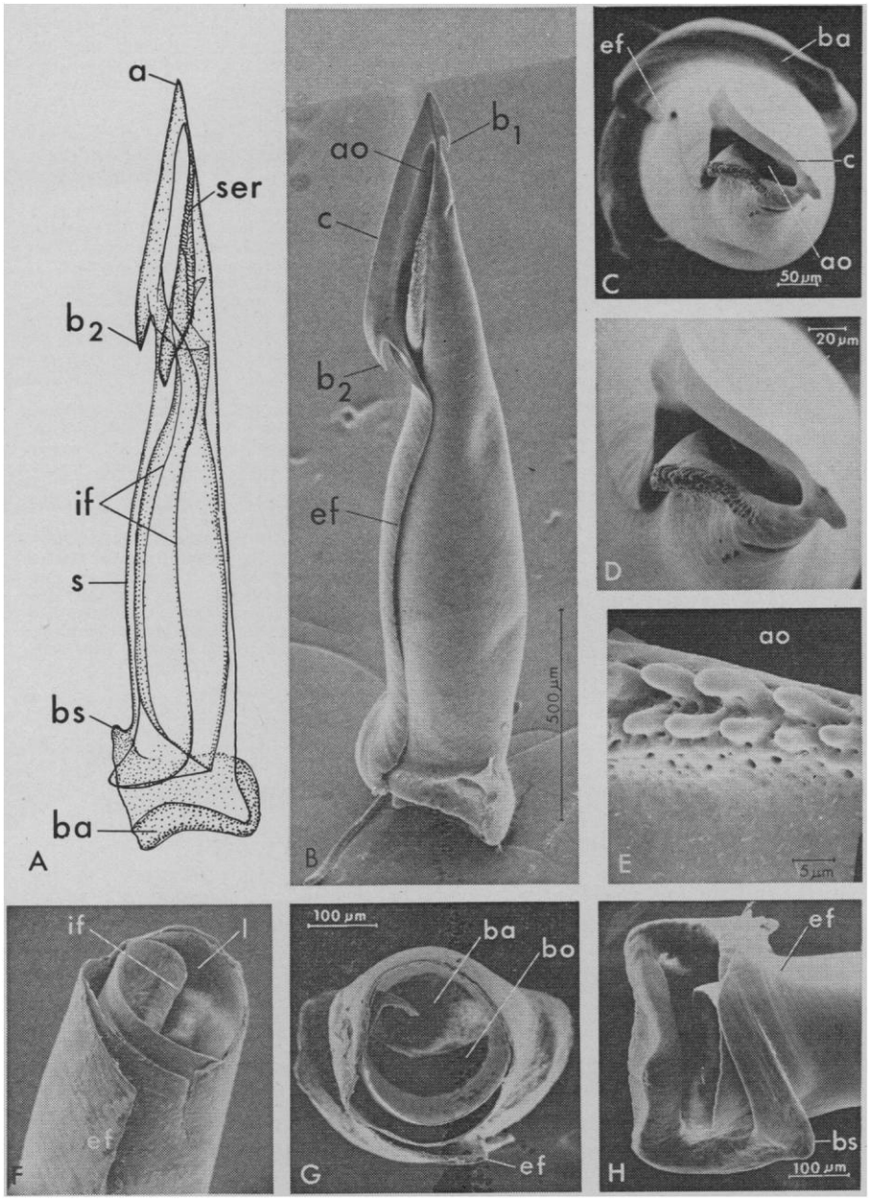

Fig. 1. Radula tooth of *Conus imperialis* Linnaeus. Views of teeth from a specimen 66 mm long from a coral reef at Cerf Island, Seychelles. (A) Camera lucida drawing of tooth as if transparent, based on light microscopy, and illustrating the difficulty in distinguishing internal and external structures: *a*, apex; b_2, second barb; *ba*, base; *bs*, basal spur; *if*, internal folds of shaft; *s*, shaft; *ser*, serration. Length of tooth, 1.85 mm. (B–H) Scanning electron micrographs. (B) Entire tooth, oriented to show adapical opening of lumen (*ao*), first and second barbs (b_1 and b_2), cutting edge (*c*), serration, and external fold of shaft (*ef*). (C) Apical view of entire tooth showing the relationship between cutting edge (*c*), adapical opening of lumen (*ao*), and serration; external fold (*ef*); and relative diameter of the base (*ba*) and shaft. (D) Enlargement of (C) emphasizing detail of the cutting edge, adapical opening, and serration. (E) Detail of denticles comprising serration and the thickened ridge from which they extend. Adapical opening of the lumen (*ao*) at top. (F) Transverse section of the shaft. The enrolled tube can be traced through 2½ whorls from the external fold (*ef*) to the internal fold (*if*), although adjacent layers are very closely applied to each other in some regions; *l*, lumen. Scale as in (G). (G) View toward the base (*ba*) of the same sectioned tooth shown in (F), showing the basal opening of the lumen (*bo*); *ef*, external fold. (H) Base of the tooth and basal portion of the shaft (*s*); view into the basal opening of the lumen; *ef*, external fold; and *bs*, basal spur.

Fully formed teeth were dissected from the short or proximal arm of the radula sheath (*3*) of preserved specimens, cleaned briefly in 1.5 percent sodium hypochlorite solution, rinsed twice in distilled water, and either freeze-dried or transferred through successively more concentrated solutions of alcohol and finally into absolute alcohol and air-dried. They were then coated with carbon followed by gold or gold-palladium in a vacuum evaporator and examined with the scanning electron microscope (Cambridge Stereoscan). For light microscopy, teeth were cleaned similarly and mounted unstained in polyvinyl lactophenol.

Figure 1, A and B, illustrates and identifies general features of the *Conus imperialis* radula tooth as seen with the light and scanning electron microscopes, respectively. Figure 1A reveals the internal folds of the tubular shaft. Within the folds is the lumen of the tooth, through which venom must pass when the tooth is charged from the venom gland and when it is injected into the prey. Figure 1B emphasizes (i) the margin of the external fold covering the inner or columellar fold; (ii) the barbs, cutting edge, and irregular double row of denticles or serration of the apical region of the tooth; and (iii) the adapical opening of the lumen, which has not previously been clearly seen. Viewing from the apex—a "worm's-eye view"—reveals the relative diameter of the base and shaft, the complexity of the serration, and the relative breadth of the adapical opening; the edge of the external fold is also seen to be curved along the length of the shaft, and the cutting edge between the apex and second barb is curved to the side (Fig. 1, C and D). Figure 1E shows details of the denticles comprising the serration.

The shaft of the tooth consists of a single roll of about 2½ whorls surrounding a large central lumen (Fig. 1, F and G). Figure 1G also indicates the relative diameter of shaft and base and shows the basal opening into the lumen.

At the base of the tooth, the outermost whorl is expanded, thickened, and strengthened. Viewed with transmitted light it is yellow, whereas the rest of the tooth appears colorless. Figure 1H shows details of the base, the large basal opening of the lumen (into which

venom flows when the tooth is charged), the basal portion of the external fold, and the end of the inner portion of the roll.

The *Conus imperialis* radula tooth is basically a sheet, presumably of chitin (*6*), rolled into a tube of 2½ whorls, the outer whorl of which is flattened and pointed at the apex and thickened and strengthened at the base. The edge of the external fold is modified into barbs and a cutting edge adapically; the adapical portion of the inner fold is reflected and thickened to form a ridge bearing complex serration (Fig. 1).

On the basis of the structure of the *Conus imperialis* tooth, the relationship of its component parts, and knowledge of the animal's food (*5*), we propose the following functions: (i) the apex, cutting edge, and serration function to penetrate the prey; (ii) the barbs and perhaps the serration catch and hold the tooth in the body of the prey; (iii) venom enters the lumen of the tooth by way of the basal opening and is conveyed into the wound via the adapical opening; and (iv) the lateral expansion of the base, its thickened nature, the basal spur, and the slight constriction of the shaft adapical to the base (Fig. 1, B and H) facilitate the action of the circular muscles of the proboscis tip; these purse just adapically of the base, firmly anchoring the tooth during the process of piercing and ingestion.

ALAN J. KOHN
Department of Zoology, University of Washington, Seattle 98195
JAMES W. NYBAKKEN
Moss Landing Marine Laboratories, Moss Landing, California 95039
JEAN-JACQUES VAN MOL
Service de Zoologie Systématique, Université Libre de Bruxelles, Belgium

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Biosynthesis of α- and β-Ecdysones from Cholesterol outside the Prothoracic Gland in Bombyx mori

Abstract. *Labeling experiments have established that cholesterol is converted into α- and β-ecdysones in isolated abdomens of silkworm larvae. Since the isolated abdomens do not contain the prothoracic glands, a doubt is cast on the long-standing principle in insect endocrinology that the prothoracic glands are the source of ecdysone secretion.*

The moulting of insects is controlled by the prothoracic glands (PTG) (*1*), which in turn are activated by the brain hormone (*2*), and ecdysone is directly responsible for the moulting (*3*). Furthermore, the PTG have been considered to be the source of ecdysone secretion, despite the lack of direct experimental evidence. Indeed, this belief was questioned by Locke (*4*) and by Weir (*5*) as a result of studies with the electron microscope and with ligation, respectively, on *Calpodes ethlius*. On the other hand, Kambysellis and Williams reported that in vitro spermatogenesis of cultured testes of *Samia cynthia* occurs only in the presence of ecdysones or endocronogically active PTG (*5*).

The following results indicate that cholesterol is converted into α- and β-ecdysones in the isolated abdomen of larvae of the silkworm (*Bombyx mori*) that are in fifth (the last) instar. Since no PTG are present in the isolated abdomens, the conjecture that the function of the PTG is ecdysone secretion should be reconsidered.

Five larvae (day 6, fifth instar) were ligated at the first abdominal segment, and the anterior parts were cut off. The isolated abdomens were each injected with 5 μc of [4-^{14}C]cholesterol in 5 μl of linolenic acid, incubated for 24 hours at 25°C, homogenized, and extracted with 80 percent ethanol. After addition of 1 mg each of unlabeled α- and β-ecdysone, the extract was concentrated to dryness, and the ecdysone fractions were collected by column chromatography through silica gel, with chloroform : methanol (4 : 1, by volume) as the solvent system (*7*).

The combined ecdysone fraction in 20 percent aqueous ethanol was submitted to the liquid chromatography technique developed for ecdysones by Hori (*8*), that is, fractionation on Amberlite XAD-2 (Rohm and Haas) with a linear gradient of 20 to 70 percent aqueous ethanol. The ecdysones were monitored by absorbance at 254 nm and by radioactivity, determined as disintegrations per minute (dpm) by liquid scintillation counting (*9*).

The combined α- and β-ecdysone fractions, which contained about 0.012 percent of the total original cholesterol radioactivity, were resubmitted to a second liquid chromatography on Poragel PN (Waters Associates) with 40 percent aqueous methanol (*10*), the ecdysones again being monitored by absorbance at 254 nm and by radioactivity (*8*). The α- and β-ecdysone fractions, about 1 mg each, were each treated with 9 mg of the respective ecdysone, and both fractions were recrystallized several times from a mixture of methanol and ethyl acetate until constant radioactivity was reached.

The final results were as follows. (i) After four recrystallizations, the yield of α-ecdysone was 7.1 mg, with radioactivity of 1.52×10^5 dpm/mmole, which corresponded to 0.00080 percent conversion of cholesterol. (Radioactivity values after two and three recrystallizations were, respectively, 1.41×10^5 and 1.67×10^5 dpm/mmole.) (ii) After five recrystallizations, the yield of β-ecdysone was 6.1 mg, with radioactivity of 3.63×10^5 dpm/mmole, which corresponded to 0.00083 percent conversion of cholersterol. (Radioactivity after the fourth recrystallization was 2.99×10^5 dpm/mmole.) The combined conversion yield of ecdysones from cholesterol was thus about 0.0016 percent and fell between the yields—0.001 and 0.015 percent—reported for two incorporation experiments with whole *Calliphora* (*11*). The low conversion yield is not surprising if one considers that cholesterol has many functions in insects (*12*).

Because of the important implications of these results, essentially the same experiments were carried out on two separate occasions with different batches of silkworms, but similar results were obtained. Furthermore, the possibility that traces of the PTG might be extending into the abdomen can be ruled out, since ligation at the first abdominal segment has been one of the standard techniques for moulting-hormone studies with *Bombyx mori*.

Identification of the two ecdysones was finally established by acetylation