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.

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### References and Notes

1. A. J. Kohn, *Proc. Nat. Acad. Sci. U.S.* **42**, 168 (1956); in *Venomous and Poisonous Animals and Noxious Plants of the Pacific Region*, H. L. Keegan and W. V. Macfarlane, Eds. (Pergamon, London, 1963), p. 83; H. Marsh, *Toxicon* **8**, 271 (1970).
2. F. H. Troschel, *Das Gebiss der Schnecken* (Nicolai's Verlag, Berlin, 1866–1893), vol. 2; A. J. Peile, *Proc. Malacol. Soc. London* **23**, 348 (1939).
3. R. Bergh, *Nova Acta Ksl. Leopoldina Carol. Deut. Akad. Naturforsch.* **65**, 67 (1895).
4. For scanning electron microscope studies of other types of gastropod radulae, see T. E. Thompson and H. E. Hinton, *Bijdr. Dierk.* **38**, 91 (1968); M. R. Carriker, *Amer. Zool.* **9**, 917 (1969); N. W. Runham, *Malacologia* **9**, 179 (1969); R. F. Thomas, *Nautilus* **84**, 118 (1971).
5. A. J. Kohn, *Ecol. Monogr.* **29**, 47 (1959) (in table 13, four specimens of *Marphysa sanguinea* actually eaten by *Conus vexillum* were erroneously included in the diet of *Conus imperialis*); J. W. Nybakken, *Veliger* **12**, 316 (1970).
6. J. Spek, *Z. Wiss. Zool.* **118**, 313 (1919).
7. Supported by NSF grant GB-17735. We thank L. Veto and A. G. Schmidt for aid with the scanning electron microscopy.

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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 $1.52 \times 10^5$ dpm/mmole, which corresponded to 0.00080 percent conversion of cholesterol. (Radioactivity values after two and three recrystallizations were, respectively, $1.41 \times 10^5$ and $1.67 \times 10^5$ dpm/mmole.) (ii) After five recrystallizations, the yield of β-ecdysone was 6.1 mg, with radioactivity of $3.63 \times 10^5$ dpm/mmole, which corresponded to 0.00083 percent conversion of cholersterol. (Radioactivity after the fourth recrystallization was $2.99 \times 10^5$ 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

followed by thin-layer chromatography. After this treatment, each ecdysone gave only two radioactive spots, corresponding to the tri- and tetraacetates of the ecdysone. Thus, the significant finding here is that $\alpha$- and $\beta$-ecdysones are formed from cholesterol in the isolated abdomen lacking the PTG.

In contrast, when labeled cholesterol was injected into larvae or isolated abdomens on day 2 of the fifth instar, a stage at which the PTG still do not show histological "activity," neither ecdysone could be detected after 24 hours. This shows that the PTG are indeed somehow related to ecdysone biosynthesis (*13*). Although it would be difficult to prove that no ecdysone is secreted from the PTG, there is no doubt that ecdysones can be synthesized outside these endocrine organs.

It should be noted that when exogenous $\alpha$-ecdysone ([23,23,24,24-tetra-$^3$H]$\alpha$-ecdysone) was injected into *B. mori* larvae at the same stage (day 6 of instar 5), more than half of the $\alpha$-ecdysone was converted into $\beta$-ecdysone within only 15 minutes (*14*), so that the relative content of $\beta$-ecdysone is larger than that of the $\alpha$ form. Thus, the equal amounts of biosynthetic $\alpha$- and $\beta$-ecdysones, as estimated from the comparable conversion yields (above), together with the rapid conversion of administered $\alpha$-ecdysone to $\beta$-ecdysone, suggest that (i) biosynthetic $\alpha$-ecdysone is not present in the free form, but rather in a bound form which is slowly hydroxylated into $\beta$-ecdysone; and (ii) the bound $\alpha$-ecdysone should be such that the ecdysone is readily liberated under conditions of extraction.

Although we have thus furnished the first clear-cut evidence for ecdysone production outside the PTG, the exact role of these glands remains enigmatic. It is possible that the so-called PTG hormone secreted from PTG into the body fluid catalyzes a step in the biosynthesis of bound $\alpha$-ecdysone from cholesterol.

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**References and Notes**

1. S. Fukuda, *J. Fac. Sci. Imp. Univ. Tokyo Sect. 4* **6**, 477 (1944); C. M. Williams, *Biol. Bull.* **93**, 89 (1947).
2. C. M. Williams, *Biol. Bull.* **103**, 120 (1952); V. B. Wigglesworth, *Nature* **169**, 558 (1951).
3. A. Butenandt and P. Karlson, *Z. Naturforsch. B* **9**, 389 (1954); C. M. Williams, *Anat. Rec.* **120**, 743 (1954); V. B. Wigglesworth, *J. Exp. Biol.* **32**, 649 (1955).
4. M. Locke, *Tissue Cell* **1**, 103 (1969).
5. S. B. Weir, *Nature* **228**, 580 (1970).
6. M. P. Kamysellis and C. M. Williams, *Science* **175**, 769 (1972).
7. S. Imai, S. Fujioka, K. Nakanishi, M. Koreeda, T. Kurokawa, *Steroids* **10**, 557 (1967).
8. M. Hori, *ibid.* **14**, 33 (1969).
9. Packard Tri-Carb model 3375; 2,5-diphenyloxazole/1,4-bis-2-(4-methyl-5-phenyloxazolyl(-benzene).
10. D. A. Schooley and K. Nakanishi, in *Modern Methods of Steroid Analysis*, E. Heftmann, Ed. (Academic Press, New York, in press); D. A. Schooley, G. Weiss, K. Nakanishi, *Steroids*, in press.
11. In the first demonstration of ecdysone biosynthesis from randomly tritiated cholesterol in *Calliphora erythrocephalia* Meig., the conversion yield was about 0.001 percent [P. Karlson and H. Hoffmeister, *Z. Physiol. Chem.* **331**, 298 (1963)]. A 0.015 percent conversion of [1-$^3$H]cholesterol into $\beta$-ecdysone in *C. stygia* has been reported [M. N. Galbraith, D. H. S. Horn, E. J. Middleton, J. A. Thompson, *Chem. Commun.* (1970), p. 179.
12. W. E. Robbins, J. N. Kaplanis, J. A. Svoboda, A. J. Thompson, *Annu. Rev. Entomol.* **16**, 53 (1971).
13. The high 7-dehydrocholesterol content of total stercids present in the PTG of the American cockroach (7 percent) (*12*) and tobacco hornworm (60 percent) (Dr. S. Chen *et al.*, unpublished results; private communication from Dr. J. N. Kaplanis, U.S. Department of Agriculture, Beltsville, Md.) has been interpreted as suggesting that the PTG is a possible site of 7-dehydrocholesterol and ecdysone biosynthesis (*12*).
14. H. Moriyama, K. Nakanishi, D. S. King, T. Okauchi, J. B. Siddall, W. Hafferl, *Gen. Comp. Endocrinol.* **15**, 80 (1970).
15. Research supported by PHS grant AI 10187. We acknowledge critical discussions with D. King (Zoecon Corp.), H. Röller (Texas A & M Univ.), J. B. Siddall (Zoecon Corp.), and C. M. Williams (Harvard Univ.) and encouragement by S. Tatsuoka (Takeda Chemical Industries). This is part XXVI of the series on insect hormones; part XXV is K. Nakanishi, D. A. Schooley, M. Koreeda, J. Dillon, *Chem. Commun.* (1971), p. 1235.

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## Length-Force Relation of Calcium Activated Muscle Fibers

Abstract. *Calcium activated skinned frog muscle fibers develop a large relative force at a sarcomere length of 1.0 micrometer. Since the normal myofilament lattice is perturbed at this length, regularity of the lattice does not appear to be an important factor in the contraction mechanism.*

Force in striated muscle fibers is developed by lateral interaction of the thick and thin myofilaments. Since the three-dimensional lattice formed by these filaments varies with fiber length, the relation between length and force provides information about the nature of the filament interaction. The most careful studies of the length-force relation have been made with electrically stimulated frog muscle fibers (*1, 2*), in which case the calcium that activates the myofilament interaction comes from the sarcoplasmic reticulum. It was discovered, however, that in electrically stimulated fibers a central core of myofibrils is not activated when sarcomeres shorten to lengths less than about 1.7 $\mu$m (*3*), which makes it difficult to interpret the length-force relation of electrically stimulated fibers at these lengths. To eliminate the effects of incomplete activation, we measured the length-force relation in skinned fibers directly activated by calcium (*4*). The main finding was that considerable force is developed at sarcomere lengths as short as 1.0 $\mu$m, even though the myofilament lattice at this length is distorted relative to that at normal sarcomere lengths.

Fibers from frog semitendinosus muscles were skinned and mounted in a force transducer according to the method of Hellam and Podolsky (*5*). Two bathing solutions were used: (i) relaxing solution containing (in millimoles per liter) KCl (140), $MgCl_2$ (1), the disodium salt of adenosine triphosphate (5), imidazole (10), and the dipotassium salt of ethyleneglycol bis(aminoethylether)tetraacetic acid (EGTA, 3) and (ii) contracting solution, in which the calcium salt of EGTA replaced the potassium salt. Both were buffered to *p*H 7. The concentration of $Ca^{2+}$ in the relaxing solution was approximately $10^{-9}M$ and in the contracting solution it was $2.5 \times 10^{-5}M$, which fully activates the myofilaments (*5*). After the initial dissection at 20°C, the fiber and experimental solutions were kept at 4°C in a thermoelectrically cooled chamber. The skinned fiber was mounted in mineral oil between two clamps (free fiber length, 1 to 5 mm) and then transferred to relaxing solution. The fiber was adjusted to a length, $l_0$, which corresponded to a striation spacing of 2.0 to 2.2 $\mu$m, as determined by a laser diffraction pattern (6328 Å). The fiber was then transferred to contracting solution and force was allowed to develop. The maximum force, $P_0$, was reached in 5 to 15 seconds. The laser pattern showed that activation changed the striation spacing in the middle of the fiber by less than 0.1 $\mu$m. After the force had reached a steady level, the