The Biosynthesis of Crustecdysone in the Blowfly *Calliphora stygia*

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Summary [1-^{3}H]Cholesterol and [1-^{3}H]-7-dehydrocholesterol are metabolised to crustecdysone in the blowfly *Calliphora stygia*.

INSECTS require[1,2] a dietary source of sterol, and some can be maintained on a diet containing cholesterol as the sole sterol. Cholesterol may thus serve as the precursor of moulting hormones in such insects. However, tritium-labelled cholesterol is reported to be incorporated into α-ecdysone in *Calliphora erythrocephala* Meig. (*C. vicina* R.-D) in only very low yield (*ca.* 0·0001%).[3] Crustecdysone is the major moulting hormone in both *C. vicina* and *C. stygia*[4] and we now find that there is significant (0·015%) incorporation of tritium-labelled cholesterol into crustecdysone in *C. stygia*.

[1-^{3}H]Cholesterol ($8{\cdot}6 \times 10^8$ d.p.m., 10c/mmole) in the form of a colloidal suspension in water containing 0·5% sodium oleate was injected into the larvae of *C. stygia* (100 animals) 6—12 hr. before puparium formation. Six hr. after puparium formation the prepupae were homogenised

and the crustecdysone isolated as previously described.[5] The isolated hormone had a total activity of $1{\cdot}4 \times 10^5$ d.p.m. When chromatographed with nonradioactive crustecdysone (400 μg) on CM-Sephadex[5] the curve of u.v. absorption plotted against elution volume coincided with that of the radioactivity. Nonradioactive crustecdysone (20 mg) was added to the crustecdysone peak fractions to give a specific activity of $3{\cdot}0 \times 10^6$ d.p.m./mmole. After several crystallizations the activity was $3{\cdot}0 \times 10^6$ d.p.m./mmole. Brief acetylation of this material afforded crustecdysone 2-acetate (specific activity $2{\cdot}9 \times 10^6$ d.p.m./mmole), which was oxidized with periodate to 2β,3β,14α-trihydroxypregn-7-en-6,20-dione 2-acetate (specific activity $2{\cdot}7 \times 10^6$ d.p.m./mmole). When the labelled crustecdysone was treated with potassium carbonate (0·04M in 90% methanol) for 24 hr. under nitrogen at 20°, conditions which promote equilibration[6] at C-5, the specific activity of the product after chromatography was $3{\cdot}2 \times 10^6$ d.p.m./mmole indicating that the radio-activity was not present in an easily easily exchangeable position.[7] While these experiments establish that cholesterol can serve as a precursor of crustecdysone in *C. stygia* the percentage incorporation of labelled cholesterol is low, possibly because of the large cholesterol pool (40 μg per animal).

Puparia of *C. stygia* were also found to contain 7-dehydrocholesterol (2 μg/animal). This sterol can support the growth of many insects[1,2] and recently it was suggested[8] as an intermediate in the biosynthesis of moulting hormones from cholesterol. To study its metabolism in *C. stygia* tritium-labelled 7-dehydrocholesterol was synthesised[9] from [1-^{3}H]cholesterol (2 mg, 250 mc/mmole) and purified by t.l.c. on silver nitrate-impregnated silica gel.[10] [1-^{3}H]-7-Dehydrocholesterol (100 μg, 250 mc/mmole) was injected into *C. stygia* larvae and the crustecdysone fraction isolated from 6 hr. prepupae as before. Again radioactivity was present in the crustecdysone isolated but the percentage incorporation (0·025%) was not significantly higher than with cholesterol. An attempt to reduce the amount of labelled cholesterol incorporated into crustecdysone by injecting nonradioactive 7-dehydrocholesterol together with the labelled cholesterol was unsuccessful. Thus, although 7-dehydrocholesterol can serve as a precursor for crustecdysone, it appears unlikely that 7-dehydrocholesterol, in a free, unconjugated form, is an intermediate in the biosynthesis of crustecdysone from cholesterol in *C. stygia*.

Work on this project was supported by a grant from the Australian Research Grants Committee.

(*Received, December 1st,* 1969; *Com.* 1824.)

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