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Sterol Biosynthesis in the Starfish *Asterias rubens* and *Henricia sanguinolenta*

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The sterols of invertebrates are often complex mixtures containing not only cholesterol, the typical sterol of higher animals, but also C_{28} and C_{29} sterols, usually regarded as phytosterols (Bergmann, 1962; Austin, 1970). The phylum Echinodermata is of particular interest, since the sterols of the Echinoidea (sea urchins), Ophiuroidea (brittle stars) and Crinoidea (feather stars) are predominantly Δ^5-sterols, whereas the Asteroidea (starfish) and Holothuroidea (sea cucumbers) contain principally Δ^7-sterols (Bergmann, 1962; Gupta & Scheuer, 1968). Previous biosynthetic studies have indicated that the sea urchin *Paracentrotus lividus* is unable to synthesize either squalene or sterols (Salaque, Barbier & Lederer, 1966) whereas a sea cucumber, *Stichopus japonicus*, was capable of squalene formation but apparently could not produce sterols (Nomura, Tsuchiya, André & Barbier, 1969). This report presents information on the sterol-synthesizing capacities of two species of starfish, *Asterias rubens* and *Henricia sanguinolenta*.

Methods. Specimens of *A. rubens and H. sanguinolenta* were maintained in aquaria kept at 10–12°C. An aqueous solution of [2-^{14}C]mevalonic acid (5–30μCi in 0.2ml) was injected into the body cavity at the base of one arm. The starfish were then kept for 17–41h in a small volume of cooled sea water, after which the non-saponifiable lipids were isolated in the usual manner. The non-saponifiable lipids were separated by t.l.c. on silica gel developed with chloroform (Goad & Goodwin, 1966). Steryl acetates were separated by t.l.c. on 10% silver nitrate–silica gel developed with cyclohexane–benzene (1:1, v/v). The squalene–thiourea adduct and squalene hexahydrochloride were prepared as described previously (Goad & Goodwin, 1966). For g.l.c. a glass column (5ft $\times \frac{1}{4}$in) packed with 3% OV-17 on 100–120-mesh Chromosorb Q was employed. The column was fitted with a splitter and samples of effluent were collected at intervals of 1min in capillary tubes at ambient temperature. Radioactivity was determined by liquid-scintillation counting (Goad & Goodwin, 1966).

Results. Incorporation of [2-^{14}C]mevalonic acid into the non-saponifiable lipids of *A. rubens* varied from 3.5 to 40%, the wide range perhaps reflecting seasonal variations or possibly the nutritional status or age of the animal. T.l.c. of the non-saponifiable lipid from a 17h incubation showed that most of the radioactivity was associated with squalene and the 4,4-dimethyl sterols, only small amounts being found in the 4-monomethyl and 4-demethyl sterols (Fig. 1). With an incubation period of 41h less radioactivity was associated with squalene but the 4-demethyl sterols still only contained 5–7% of the radioactivity of the non-saponifiable lipid (Fig. 1).

Radioassay of the effluent from a g.l.c. analysis of the squalene fraction showed that radioactivity coincided exactly with the squalene mass peak. The incorporation of [2-^{14}C]mevalonic acid into squalene was confirmed by rechromatography of the squalene fraction [on silica gel, developed with ethyl acetate–light petroleum (b.p. 40–60°C), 1:20, v/v], dilution with authentic squalene to give a specific radioactivity of 1.30×10^5d.p.m./mmol and purification through the thiourea adduct (1.39×10^5d.p.m./mmol), followed by formation of the hexahydrochloride (1.13×10^5, 1.22×10^5, 1.05×10^5, 1.03×10^5 and 1.15×10^5d.p.m./mmol for successive crystallizations).

An examination of the non-saponifiable lipid of *A. rubens* showed the presence of a small quantity (0.15%) of a 4,4-dimethyl sterol mixture, which was acetylated and separated into three fractions (R_F 0.39, 0.35 and 0.32 respectively) by t.l.c. on silver nitrate–silica gel. The constituent compounds of these fractions were identified by g.l.c. and mass spectrometry as cycloartanyl acetate [mass spectrum: *m/e* 470 (M^+), 455 (M^+-CH_3), 410 (M^+-acetate), 297 (M^+-side chain$-$acetate), 288 (M^+-ring A)], cycloartenyl acetate [*m/e* 468 (M^+), 453 (M^+-CH_3), 408 (M^+-acetate), 297 (M^+-side chain$-$acetate), 286 (M^+-ring A)], lanosteryl acetate [*m/e* 468 (M^+), 453 (M^+-CH_3), 408 (M^+-acetate)], 4,4-dimethyl-5α-cholesta-8,24-dien-3β-yl acetate [*m/e* 454 (M^+), 439 (M^+-CH_3), 394 (M^+-acetate), 341 (M^+-side chain$-$2H)] and 4,4-dimethyl-5α-cholesta-7,24-dien-3β-yl acetate [*m/e* 454 (M^+), 439 (M^+-CH_3), 394 (M^+-acetate), 341 (M^+-side chain$-$2H)]. When the radioactive 4,4-dimethyl sterols from an incubation with [2-^{14}C]mevalonic acid were acetylated and subjected

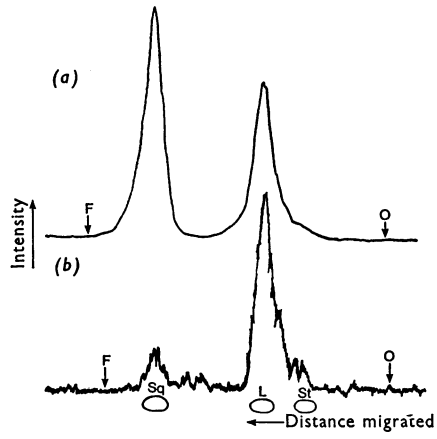

Fig. 1. Thin-layer radioscan of the non-saponifiable lipids isolated from *Asterias rubens* after incubation with [2-^{14}C]mevalonic acid for (*a*) 17h and (*b*) 41h. For experimental details see the text. Abbreviations: O, origin; F, solvent front; St, sterol; L, lanosterol; Sq, squalene.

to t.l.c. on silver nitrate–silica gel, radioautography showed that very little radioactivity was associated with the cycloartanyl acetate band (R_F 0.39). The band (R_F 0.35) containing lanosteryl acetate and cycloartenyl acetate, which co-chromatograph, was moderately labelled, whereas the most polar band (R_F 0.32), corresponding to the acetates of 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol and its Δ^7 isomer, was heavily labelled. G.l.c. of the lanosteryl acetate–cycloartenyl acetate fraction showed that the only radioactive peak coincided with the lanosteryl acetate mass peak; negligible radioactivity was associated with the cycloartenyl acetate peak. The labelling of lanosteryl acetate was confirmed by addition of carrier material and crystallization to constant specific radioactivity. Examination of the labelled 14-demethyl compounds by g.l.c. showed that radioactivity was present in both 4,4-dimethyl-5α-cholesta-8,24-dien-3β-yl acetate and 4,4-dimethyl-5α-cholesta-7,24-dien-3β-yl acetate, with the former compound slightly more heavily labelled.

G.l.c.–mass spectrometry of the 4-demethyl sterols of *A. rubens* showed a mixture of Δ^7-sterols of which the major compound (approx. 75%) was 5α-cholest-7-en-3β-ol [mass spectrum: *m/e* 386 (M^+), 371 (M^+-CH_3), 353 ($M^+-CH_3-H_2O$), 273 (M^+–side chain), 255 (M^+–side chain$-H_2O$), 213 (M^+–side chain$-H_2O-42$)]. Analysis by g.l.c. of a sample of the 4-demethyl sterols labelled from [2-^{14}C]mevalonic acid showed that the major radioactive component was 5α-cholest-7-en-3β-ol, but there was evidence that radioactivity was also present in other unidentified compounds that were possibly unstable under the g.l.c. conditions employed. When a sample of the radioactive 4-demethyl sterols was purified by silver nitrate–silica gel t.l.c. and added to pure 5α-cholest-7-en-3β-ol, crystallization to constant specific radioactivity was achieved (128, 124, 120, 120 and 120 d.p.m./mg for successive recrystallization), showing that *A. rubens* is capable of synthesizing 4-demethyl sterols.

In a similar experiment with the starfish *H. sanguinolenta* [2-^{14}C]mevalonic acid was incorporated during a 24h incubation into squalene and into the 4,4-dimethyl sterol fraction. Further examination of the 4,4-dimethyl sterols, however, showed a distribution of radioactivity different from that found in *A. rubens*. In addition to lanosterol there was also substantial label in 24,25-dihydrolanosterol, but the 14-demethyl compounds contained relatively much less radioactivity. The incorporation of [2-^{14}C]mevalonic acid into the 4-monomethyl and 4-demethyl sterols of *H. sanguinolenta* was extremely low and identification of the labelled 4-demethyl sterol component(s) was not possible.

Discussion. The starfish *Pisaster ochraceus* has been shown to convert dietary cholest-5-en-3β-ol into 5α-cholest-7-en-3β-ol (Fagerlund & Idler, 1960) and we have since demonstrated that this conversion also occurs in *A. rubens* and *Solaster papposus*, possibly via the intermediacy of 5α-cholestan-3β-ol (Smith & Goad, 1971). The ability of *A. rubens* to synthesize 5α-cholest-7-en-3β-ol, if only to a very limited extent, from mevalonic acid is demonstrated by the present work. It thus appears that in starfish the sterols can arise both from synthesis *de novo* and by suitable modification of dietary sterols. The high incorporation of [2-^{14}C]-mevalonic acid into squalene and 4,4-dimethyl sterols compared with the amount of radioactivity appearing in the 4-demethyl sterols, however, suggests the possibility of divergent biosynthetic pathways from the 4,4-dimethyl sterols. For example, they may be metabolized either to give 5α-cholest-7-en-3β-ol or alternatively to produce the steroidal glycosides (asterosaponins) that occur in the Asteroidea (Mackie & Turner, 1970).

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Austin, J. (1970). *Adv. Steroid Biochem. Pharmac.* **1**, 73.
Bergmann, W. (1962). In *Comparative Biochemistry*, vol. 3, p. 144. Ed. by Florkin, M. & Mason, H. S. New York and London: Academic Press.
Fagerland, U. H. M. & Idler, D. R. (1960). *Can. J. Biochem. Physiol.* **38**, 997.

Goad, L. J. & Goodwin, T. W. (1966). *Biochem. J.* **99**, 735.
Gupta, K. C. & Scheuer, P. J. (1968). *Tetrahedron*, **24**, 5831.
Mackie, A. M. & Turner, A. B. (1970). *Biochem. J.* **117**, 543.
Nomura, T., Tsuchiya, Y., André, D. & Barbier, M. (1969). *Bull. Jap. Soc. scient. Fish.* **35**, 299.
Salaque, A., Barbier, M. & Lederer, E. (1966). *Comp. Biochem. Physiol.* **19**, 45.
Smith, A. G. & Goad, L. J. (1971). *FEBS Lett.* **12**, 233.