THE METABOLISM OF CHOLESTEROL BY THE ECHINODERMS *ATERIAS RUBENS* AND *SOLASTER PAPPOSUS*

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1. Introduction

Examination of the sterols of various echinoderms has revealed that while the sea-urchins (Echinoidea) and brittle-stars (Ophiuroidea) contain Δ^5-sterols the starfish (Asteroidea) and sea-cucumbers (Holothuroidea) contain, by contrast, predominantly Δ^7-sterols [1–4]. Investigations on the sterol biosynthetic capacities of these invertebrates showed that a sea-urchin, *Paracentrotus lividus,* was apparently unable to make either squalene or sterols [5]. The sea-cucumber, *Stichopus japonicus,* did not produce sterol but was capable of limited squalene biosynthesis and it was suggested that the sterols of this animal may be of dietary origin [6]. In two species of starfish, *Asterias rubens* and *Henricia sanguinolenta,* squalene and lanosterol were rapidly labelled from 2-^{14}C-mevalonic acid but cholest-7-enol, the major sterol, was relatively poorly labelled [4, 7]. Since it was previously indicated that the starfish. *Pisaster ochraceus,* can convert dietary cholest-5-enol into cholest-7-enol [8] we have reinvestigated this problem and now report the results obtained with the starfish *A. rubens* and *Solaster papposus.*

2. Materials and methods

4-^{14}C-Cholest-5-enol was purchased from the Radiochemical Centre, Amersham. 4-^{14}C-5α-Cholestanol was prepared by reduction of 4-^{14}C-cholest-5-enol [9]. *A. rubens* and *S. papposus* were maintained in sea water aquaria held at 10–12°.

2.1. *Administration of sterols to starfish*

An emulsion of the radioactive sterol in 0.2 ml of aqueous 5% Tween 80 was injected into the body cavity at the base of one leg.

2.2. *Isolation of sterols*

The non-saponifiable lipids were obtained in the usual manner and the total sterol mixture isolated by thin layer chromatography (TLC) on silica gel developed with chloroform.

5α-Cholestanol and cholest-7-enol, which co-chromatograph, were separated from cholest-5-enol by TLC on $AgNO_3$-silica gel developed with chloroform. Alternatively 5α-cholestanol was separated from both cholest-5-enol and cholest-7-enol by conversion of the latter two compounds into the corresponding epoxides by treatment of the sterol mixture with a molar quantity of *m*-chloroperbenzoic acid in chloroform for 3 hr. TLC on silica gel developed with ethyl acetate:chloroform (35:65) gave a good separation of 5α-cholestanol (R_f = 0.66) 7α, 8α-epoxy-5α-cholestanol (R_f = 0.52) and 5α, 6α-epoxy-cholestanol (R_f = 0.45).

Gas–liquid chromatography employed a 5 ft column of 1% SE-30 on 100–120 mesh silanised Chromosorb P at 220°. Samples were trapped at ambient temperature in glass capillary tubes at one minute intervals and assayed for radioactivity by liquid scintillation counting.

3. Results

4-^{14}C-Cholest-5-enol (5 μCi) was injected into a specimen of *A. rubens* and the sterols isolated after

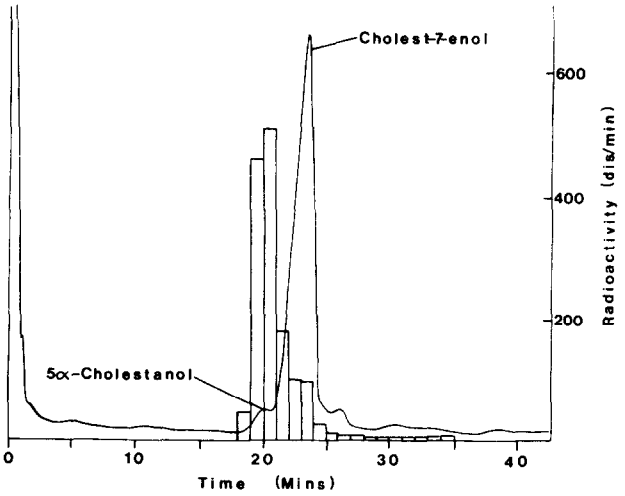

Fig. 1. Gas–liquid chromatography of the Δ^7 and saturated sterol fraction isolated from *A. rubens* after injection of 4-^{14}C-cholest-5-enol.

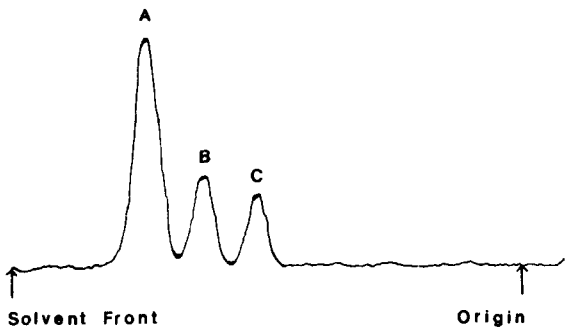

Fig. 2. Thin-layer radioscan after epoxidation of the sterols isolated from *A. rubens* following injection of 4-^{14}C-cholest-5-enol. A) 5α-Cholestanol; B) 7α, 8α-epoxycholestanol; C) 5α, α-epoxycholestanol.

41 hr incubation. Following removal of unchanged 4-^{14}C-cholest-5-enol by $AgNO_3$-silica gel TLC the remaining mixture of Δ^7 and saturated sterols was added to carrier cholest-7-enol. Crystallisation resulted in a large drop in specific activity from an initial value of 548 dpm/mg to a constant value of 80 dpm/mg showing that although some conversion of cholest-5-enol into cholest-7-enol had occurred the bulk of the radioactivity was present in another sterol product. Gas–liquid chromatography of a portion of the Δ^7-sterol and saturated sterol mixture revealed that this radioactive metabolite had a retention time corresponding to 5α-cholestanol (fig. 1).

In a second experiment a starfish (*A. rubens*) was injected with 4-^{14}C-cholest-5-enol (10 μCi) and the animal kept for seven days before isolation of the sterols. Formation of the sterol epoxides followed by TLC again showed that the major radioactive component was 5α-cholestanol and that the epoxide of cholest-7-enol was also appreciably labelled (fig. 2). Crystallisation of these compounds with carrier material to constant specific activity confirmed the conversion of cholest-5-enol into both 5α-cholestanol and cholest-7-enol (table 1). Similar results were obtained following incubation of *Solaster papposus* with 4-^{14}C-cholest-5-enol for nine days (table 1).

Table 1

Crystallisation of the 5α-cholestanol and 7α, 8α-epoxycholestanol obtained from *A. rubens* and *S. papposus* after injection of 4-^{14}C-cholest-5-enol.

	7α, 8α-Epoxycholestanol		5α-Cholestanol	
	A. rubens	*S. papposus*	*A. rubens*	*S. papposus*
Initial	4537*	656	2681	1227
1st Crystallisation	4491	722	2410	1230
2nd	4558	699	2524	1211
3rd	4720	664	2564	1269
4th	4885	655	2534	1240

* dpm/mg

The possible intermediacy of 5α-cholestanol in the conversion of cholest-5-enol into cholest-7-enol was examined by incubation of 4-^{14}C-5α-cholestanol (2.75 μCi) with *A. rubens* for three days. A thin layer radioscan of the epoxidised sterols showed that the cholest-7-enol epoxide was labelled and constituted about 18% of the recovered radioactive sterol. This was confirmed by elution of the 7α, 8α-epoxycholestanol and crystallisation with carrier material to constant specific activity (first crystallisation 643 dpm/mg; fifth crystallisation 645 dpm/mg).

4. Discussion

These experiments confirm and extend the earlier work [8] showing that starfish can convert cholest-5-enol into cholest-7-enol. The high conversion of cholest-5-enol into 5α-cholestanol suggests that the latter compound may be an intermediate in this conversion and this is further indicated by the demonstration that *A. rubens* can convert 5α-cholestanol into cholest-7-enol. Since the present experiments were conducted with whole animals the possibility that the observed sterol transformations were mediated by micro-organisms present in the digestive tract must be considered. However it is notable that the transformation of 5α-cholestanol into cholest-7-enol by insects has been reported [10, 11] and the possible intermediacy of saturated sterols in the conversion of Δ^5 sterols into Δ^7 sterols by insects has been considered [12].

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