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Sterol Biosynthesis by the Sea Urchin *Echinus esculentus*

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1. The 4-demethyl sterols of *Echinus esculentus* consisted of cholesterol as the major component, with lower concentrations of nine other C_{26}, C_{27}, C_{28} and C_{29} Δ^5 sterols. 2. [2-^{14}C]Mevalonic acid was readily incorporated by the urchin into squalene, lanosterol and desmosterol but only to a small extent into cholesterol. 3. [26-^{14}C]Desmosterol did not appear to be reduced to give cholesterol, but conversion of 5α-[2-$^3H_2$]lanost-8-en-3β-ol into cholesterol was observed. 4. No C-24 dealkylation of [4-^{14}C]sitosterol or metabolism of [4-^{14}C]cholesterol could be detected.

The sterol biosynthetic capabilities of members of the phylum Echinodermata are now under active investigation (Goad *et al.*, 1972; Voogt, 1973; Grossert, 1972). The starfish (Asteroidea), which possess mainly Δ^7 sterols (Bergmann, 1962; Goad *et al.*, 1972), are capable of both synthesizing 5α-cholest-7-en-3β-ol *de novo* and producing this compound from dietary cholesterol (Fagerlund & Idler, 1960; Smith & Goad, 1971*a,b*). The sea cucumbers (Holothuroidea), the other class of echinoderms which contain Δ^7 sterols (Bergmann, 1962; Gupta & Scheuer, 1968; Nomura *et al.*, 1969*a*; Goad *et al.*, 1972), probably obtain these compounds by similar methods (Goad *et al.*, 1972; Voogt & Over, 1973). However, Nomura *et al.* (1969*b*) could show no conversion of [1,2-^{14}C]acetate into Δ^7 sterols by the species *Stichopus japonicus*, although squalene was labelled. Among the other three classes of echinoderm, which all possess Δ^5 sterols (Bergmann, 1962; Gupta & Scheuer, 1968), biosynthetic investigations on the sea lilies (Crinoidea) have not to our knowledge been reported, but the brittle stars (Ophiuroidea) do appear to synthesize some 3β-sterols from [2-^{14}C]mevalonic acid (Goad *et al.*, 1972; Rubinstein, 1973) and [1-^{14}C]acetate (Voogt, 1973). Three reports have now appeared on the fifth class of the Echinodermata, the sea urchins (Echinoidea). Salaque *et al.* (1966) could not detect any biosynthesis of 3β-sterol or squalene from [1,2-^{14}C]acetate by the species *Paracentrotus lividus*. In contrast Voogt (1972) has shown that *P. lividus* and two other species of sea urchin could produce squalene and 3β-sterols from [1-^{14}C]acetate. Since the report of Salaque *et al.* (1966) we have reported briefly (Goad *et al.*, 1972) that the species *Echinus esculentus* could metabolize [2-^{14}C]mevalonic acid to squalene, lanosterol and desmosterol (cholesta-5,24-dien-3β-ol). The work described here concerns the identification of the 4-demethyl sterols of *E. esculentus* and incubation of this species with (3*RS*)-[2-^{14}C]-mevalonic acid, [2-$^3H_2$]5α-lanost-8-en-3β-ol, [4-^{14}C]-cholesterol, [4-^{14}C]sitosterol and [26-^{14}C]desmosterol.

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Experimental

Materials

Lanosterol and 5α-lanost-8-en-3β-ol were gifts from Dr. E. Ghisalberti (Department of Biochemistry, University of Liverpool). Desmosterol (m.p. 119–121°C) was extracted from *Rhodymenia palmata* (Gibbons *et al.*, 1967) and also given by Dr. H. H. Rees (Department of Biochemistry, University of Liverpool). (3*RS*)-[2-^{14}C]Mevalonic acid (10mCi/mmol), [4-^{14}C]sitosterol (61 mCi/mmol), [26-^{14}C]-desmosterol (53 mCi/mmol) and [4-^{14}C]cholesterol (59mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. 5α-[2-$^3H_2$]Lanost-8-en-3β-ol (6.4mCi/mmol) was prepared by ^3H_2O base-catalysed exchange labelling (Lenton *et al.*, 1971) of 58mg of 5α-lanost-8-en-3-one [m.p. 116–119°C; lit. m.p. 119.5–120.5°C (Ruzicka *et al.*, 1945); *m/e* 442 (M^+); i.r. 1730cm^{-1}, carbonyl]. The labelled ketone was reduced with $LiAlH_4$ and the 3β-hydroxy epimer isolated by t.l.c. on silica gel developed with chloroform (48mg; 15μCi/mg). Dilution with authentic 5α-lanost-8-en-3β-ol and repeated crystallizations showed no decrease in specific radioactivity. The purity was further established by preparative g.l.c. and t.l.c. of the acetate $AgNO_3$-impregnated silica gel on (performed by Dr. G. H. Beatstall).

Methods

The Liebermann–Burchard test was performed as described by Kemp *et al.* (1967). G.l.c. was performed as described by Lenton *et al.* (1971) by using 1% SE-30 on Gas-Chrom Q as the stationary phase. For g.l.c.–mass spectrometry a Pye 104 gas chromatograph was coupled via a Biemann separator to an AEI MS12 instrument (Smith *et al.*, 1973).

Animals for incubation were injected in the area beside the operculum with (3*RS*)-[2-^{14}C]mevalonic acid dissolved in 0.2ml of water. Sterols for injection were emulsified in 0.2ml of 5% (w/v) Tween 80

solution. During incubations the sea urchins were maintained in 5 litres of aerated sea water at 10–12°C. They were killed by freezing, saponified in ethanolic 10% (w/v) KOH at 60°C and the non-saponifiable lipids extracted into diethyl ether–light petroleum (b.p. 40–60°C) mixture (1:1, v/v). Separations of non-saponifiable lipids into squalene, 4,4-dimethyl sterols, 4-monomethyl sterols and 4-demethyl sterols were accomplished by t.l.c. on silica gel G (E. Merck, Darmstadt, Germany) developed with chloroform (Goad & Goodwin, 1966). 4-Demethyl sterols and 4,4-dimethyl steryl acetates were separated into further fractions by t.l.c. on 10% (w/w) $AgNO_3$-impregnated silica gel G plates. Sterols were developed by triple development with chloroform and 4,4-dimethyl steryl acetates with cyclohexane–benzene (1:1, v/v).

Sterol mixtures were brominated by the method of Fieser (1955). After repeated washings with diethyl ether the Δ^5 sterols were regenerated by refluxing the bromides with zinc powder in diethyl ether. Sterols and steryl acetates were crystallized from chloroform–methanol. Cholesterol was epoxidized with *m*-chloroperbenzoic acid (Smith & Goad, 1971*a*) and the epoxide crystallized from acetone.

Radioactivity was determined by using a Beckmann LS200B liquid-scintillation counter (Lenton *et al.*, 1971). Radioscanning of t.l.c. plates was accomplished with a Panax radioscanner and radioautograms were obtained by exposure to Kodak Kodirex X-ray film. The distribution of radioactivity during g.l.c. was measured by dividing the column effluent with a 9:1 splitter and trapping the major portion in glass capillaries at ambient temperature. The sterols were then washed into scintillation vials by using diethyl ether and assayed for radioactivity.

Isolation of the 4-demethyl sterols of E. esculentus. A specimen of *E. esculentus* (305g) yielded 35mg of non-saponifiable lipid, which when examined by t.l.c. gave 18mg of 4-demethyl sterols. The crude sterol mixture showed the green colour with the Liebermann–Burchard reagent associated with Δ^5 sterols (Moore & Baumann, 1952). When analysed by g.l.c. (1% SE-30) seven peaks were observed and the major component (peak C) had the retention time of cholesterol. G.l.c.–mass spectrometry of the sterols as their trimethylsilyl ether derivatives allowed their further identification (Eneroth *et al.*, 1964, 1965; Brooks *et al.*, 1968; Knights, 1967; Wyllie & Djerassi, 1968) as shown in Table 1. All sterols gave ions at *m/e* 129 and complementary ions at M^+-129, derived by loss of C-1, C-2 and C-3 from ring A plus trimethylsilanol (Me_3SiOH), a fragmentation which seems to be restricted to Δ^5 sterols (Eneroth *et al.*, 1965; Brooks *et al.*, 1968). The C_{26} sterol (peak A) has been identified in previous work as 26, 27-dinorergosta-5,22-dien-3β-ol (Goad *et al.*, 1972) and was characterized by ions *m/e* 442 (M^+), 352 (M^+-Me_3SiOH), 337 ($M^+-Me_3SiOH-CH_3$), 313 (M^+-129) and 255(M^+-side chain$-$MeSiOH). The Δ^{22} bond was revealed by the fission of the C-20–C-21 bond to give an ion *m/e* 372 (Eneroth *et al.*, 1965; Lenfant *et al.*, 1967). A small quantity of cholesta-5,22-dien-3β-ol was observed (peak B) and also present were the 24-methyl and 24-ethyl homologues, 24-methylcholesta-5,22-dien-3β-ol (peak D) and 24-ethylcholesta-5,22-dien-3β-ol (peak F). Peak D also contained a second component, cholesta-5,24-dien-3β-ol, the trimethylsilyl ether giving diagnostically important ions at 456 (M^+), 343 (M^+-side chain$-$2H), 327 (M^+-129) and in particular an ion at *m/e* 69 known to be formed by loss of isoprene from Δ^{24} sterols (Galli & Maroni, 1967; Brooks *et al.*, 1968). Peak E consisted of two components, the mass spectra of the trimethylsilyl ethers showing them to be 24-methylcholesta-5-en-3β-ol (M^+ at *m/e* 472) and

Table 1. *G.l.c. and mass-spectral data for the trimethylsilyl ethers of the 4-demethyl sterols of E. esculentus*

For details see the text. Key: a, M^+-Me_3SiOH; b, M^+-side chain; c, M^+-side chain$-$2H; d, M^+-side chain$-Me_3SiOH$; e, M^+-129; f, M^+-(C-22 to C-29)$-$H; g, M^+-(C-23 to C-29)$-$H; h, M^+-(C-23 to C-29)$-$H$-Me_3SiOH$.

Sterol	G.l.c. peak	Relative retention time	Percentage composition	Mass-spectral fragmentation ions								
				M^+	a	b	c	d	e	f	g	h
26,27-Dinorergosta-5,22-dien-3β-ol	A	0.59	1.8	442	352	—	343	255	313	372	—	—
Cholesta-5,22-dien-3β-ol	B	0.89	2.9	456	366	345	343	255	327	372	—	—
Cholesterol	C	1.00	78.5	458	368	345	—	255	329	—	—	—
Cholesta-5,24-dien-3β-ol	D	1.10	8.4	456	366	—	343	255	327	372	—	—
24-Methylcholesta-5,22-dien-3β-ol				470	380	—	343	255	341	372	—	—
24-Methylenecholest-5-en-3β-ol	E	1.32	4.0	470	380	—	343	255	341	372	386	296
24-Methylcholest-5-en-3β-ol				472	382	—	—	255	343	—	—	—
24-Ethylcholesta-5,22-dien-3β-ol	F	1.46	0.8	484	394	—	343	255	355	372	—	—
24-Ethylidenecholest-5-en-3β-ol	G	1.79	3.6	484	394	—	343	255	355	372	386	296
24-Ethylcholest-5-en-3β-ol				486	396	—	—	255	357	—	—	—

24-methylenecholest-5-en-3β-ol (M^+ at m/e 470). The latter sterol was distinguished from the $\Delta^{5,22}$ isomer by the ions at m/e 386 [M^+−(C-23 to C-28)−H] and 296 [M^+−(C-23 to C-28)−H−Me_3SiOH] (Knights, 1967). Similarly peak G contained two sterols, 24-ethylcholest-5-en-3β-ol (M^+ at m/e 486) and 24-ethylidenecholest-5-en-3β-ol (M^+ at m/e 484), the $\Delta^{24(28)}$ bond in the latter compound being revealed by the ions at m/e 386 and 296 (Knights, 1967). The stereochemistry at C-24 of the saturated side-chain-alkylated sterols could not be determined by g.l.c. or mass spectrometry. Similarly the configuration of the $\Delta^{24(28)}$ sterol (i.e. Z or E isomer) was not resolved since heterogeneity of the g.l.c. peaks did not allow the accurate measurement of retention time needed to distinguish between the two C_{29} isomers (Brooks *et al.*, 1968).

Metabolism of [2-^{14}*C*]*mevalonic acid.* Two specimens of *E. esculentus* were incubated with 5μCi of [2-^{14}C]mevalonic acid for 41h and 24h (Expts. A and B respectively). The non-saponifiable lipids were then extracted and the incorporations of radioactivity determined (Table 2). Radioscanning of the thin-layer chromatogram of a portion of the lipid from Expt. A (Fig. 1) showed that the radioactivity was distributed mainly between squalene (R_F 0.8), 4,4-dimethyl sterols (R_F 0.32), 4-monomethyl sterols (R_F 0.27) and 4-demethyl sterols (R_F 0.21). Two other peaks of radioactivity were also observed (R_F 0.73 and 0.58). The lipids were then separated preparatively into squalene, 4,4-dimethyl, 4-monoethyl and 4-demethyl sterol fractions by t.l.c. (Table 2).

The squalene fractions were diluted with authentic material (Expt. A, 158.2mg; Expt. B, 102.7mg), purified through formation of the thiourea adduct and then converted into the hexahydrochloride (Goad & Goodwin, 1966) and crystallized from acetone to constant specific radioactivity (Table 3).

The 4,4-dimethyl sterols were acetylated and subjected to t.l.c. on $AgNO_3$-impregnated silica gel. A radioautogram of the 4,4-dimethyl steryl acetates from Expt. A showed that no radioactivity chromatographed with 24,25-dihydro-4,4-dimethyl steryl acetates. Approximately 25% of the radioactivity had an R_F value which corresponded to that of lanosteryl acetate (R_F 0.33), but this substance did not completely separate from the Δ^{24}-14-demethyl-4,4-dimethyl sterols (R_F 0.29), which contained the remainder of the radioactivity. The proportion of radioactivity co-chromatographing with lanosteryl acetate was higher in Expt. B. The lanosteryl acetate fractions from both experiments were obtained by preparative t.l.c. but were thought to contain contaminating 14-demethyl-4,4-dimethyl compounds. This was confirmed by crystallization with authentic lanosteryl acetate (Expt. A, 61mg; Expt. B, 117mg). The specific radioactivities became constant only after substantial decreases (Expt. A, 435, 228, 205, 151, 135, 130, 125, 119, 120d.p.m./mg; Expt. B, 256, 192, 178, 180, 182d.p.m./mg for successive crystallizations).

When the 4-demethyl sterols were examined by t.l.c. on $AgNO_3$-impregnated silica gel the radioautograms revealed that very little radioactivity chromatographed with the R_F of cholesterol or the 24-alkylated sterols. However, at least six unidentified radioactive compounds were detected. A portion of the sterols from Expt. A (46000d.p.m.; 10.8mg) was diluted with cholesterol (100mg) and crystallized repeatedly. The specific radioactivity only approached a constant value after an 80% decrease (451, 373, 232, 187, 160, 134, 92, 95d.p.m./mg for successive crystallizations).

Another portion of the sterols from Expt. A was separated by t.l.c. on $AgNO_3$-impregnated silica gel into a cholesterol fraction (14700 d.p.m.), which was again diluted with carrier (41.4mg) and crystallized. The specific radioactivity was constant after an initial decrease (227, 119, 96, 70, 59, 63d.p.m./mg for successive crystallizations). When a portion of the 4-demethyl sterols (52000d.p.m.) was purified through formation of the 5,6-dibromide and the cholesterol regenerated and crystallized, the specific radioactivity also fell (139, 62, 53, 44, 40d.p.m./mg for successive crystallizations).

The t.l.c. of the 4-demethyl sterols on $AgNO_3$-impregnated silica gel also yielded a band chromatographing with desmosterol (Expt. A 12200d.p.m.; Expt. B 10750d.p.m.) which was diluted with authentic carrier sterol (Expt. A, 23.6mg; Expt. B, 28.2mg) and crystallized to a constant specific radioactivity (Expt. A, 456, 308, 313, 330, 328d.p.m./mg; Expt. B, 310, 261, 263, 263, 248, 256d.p.m./mg for successive crystallizations).

Metabolism of 5α-[2-$^3H_2$]*lanost-8-en-3β-ol.* An *E. esculentus* (312g) was injected with 17.8μCi of

Table 2. *Incubation of E. esculentus with* [2-^{14}*C*]*mevalonic acid*

Experimental details are described in the text.

	Expt. A (October 1969)	Expt. B (October 1970)
Time of incubation (h)	41	24
Non-saponifiable lipid (mg)	34.1	149.5
Weight of 4-demethyl sterols (mg)	11.4	92.0
Percentage incorporation of ^{14}C into the non-saponifiable lipid	13.8	7.6
Radioactivity (d.p.m.) incorporated into:		
Squalene	55900	20800
4,4-Dimethyl sterols	55500	75800
4-Monomethyl sterols	74900	48000
4-Demethyl sterols	186000	108000

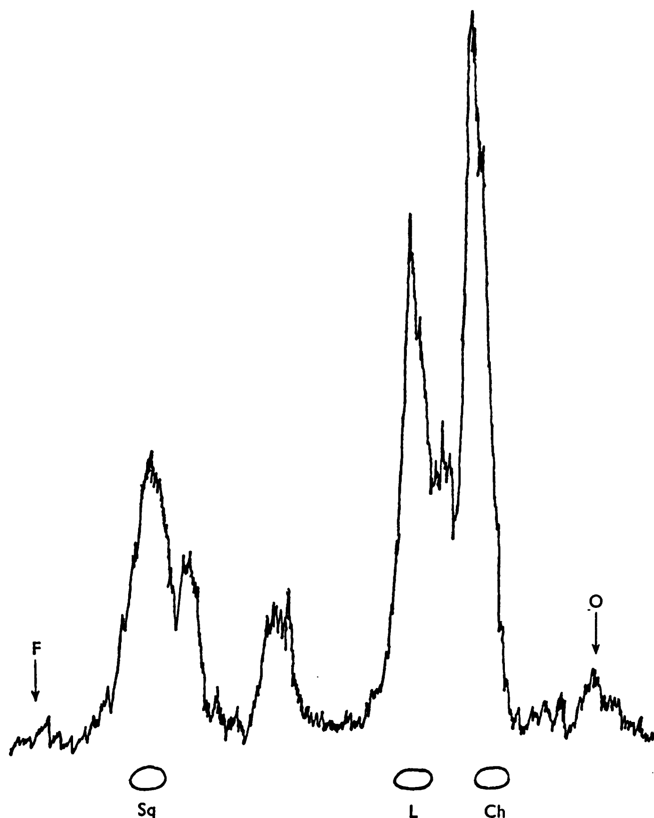

Fig. 1. *Thin-layer radiochromatogram of the non-saponifiable lipid isolated from E. esculentus after incubation with* [2-^{14}C]*mevalonate*

An animal was injected with [2-^{14}C]mevalonate and the non-saponifiable lipid extracted after 41 h incubation. A portion of the radioactive non-saponifiable lipid was separated by t.l.c. on silica gel developed with chloroform and the plate scanned for radioactivity. Marker compounds were: Sq. squalene; L, lanosterol; Ch, cholesterol; O is origin and F the solvent front.

Table 3. *Analysis of the labelled squalene samples isolated from E. esculentus after incubation with* [2-^{14}C]*mevalonic acid*

Experimental details are described in the text.

	10^{-7} × Specific radioactivity (d.p.m./mol)	
	Expt. A	Expt. B
Labelled squalene after dilution with carrier squalene	10.7	4.34
After purification via the squalene–thiourea adduct	13.0	4.76
Squalene hexahydrochloride:		
1st crystallization	9.2	5.29
2nd crystallization	9.1	5.08
3rd crystallization	10.8	5.08

5α-[2-^{3}H$_2$]lanost-8-en-3β-ol and incubated for 6 days. The non-saponifiable lipids (197.1 mg; 13.6 μCi) yielded 40.7 mg of 4-demethyl sterol (184200 d.p.m.). A portion of the 4-demethyl sterols (11.4 mg; 51600 d.p.m.) was diluted with authentic cholesterol (99.2 mg), purified via the formation of the dibromide and then crystallized repeatedly. The specific radioactivity became constant after an initial decrease after dibromide formation (172, 138, 134, 128, 115, 129 d.p.m./mg for successive crystallizations). Another portion (25.9 mg; 121000 d.p.m.) was purified by t.l.c. on AgNO$_3$-impregnated silica gel (17.7 mg; 50200 d.p.m.) and then one-half was diluted with cholesterol (32.5 mg). The specific radioactivity became constant during repeated crystallizations (609, 427, 402, 411, 397 d.p.m./mg for successive crystallizations). The remaining half was

also diluted with cholesterol (30.6mg), converted into 5α,6α-epoxycholestan-3β-ol and purified by t.l.c. (Smith & Goad, 1971*a*) to yield 37.7mg of the epoxide (15600d.p.m.), which was then crystallized to constant specific radioactivity (415, 410, 447, 417, 440d.p.m./mg for successive crystallizations).

Incubation with [26-^{14}C]desmosterol. A specimen of *E. esculentus* was incubated for 6 days with 2μCi of [26-^{14}C]desmosterol and the 4-demethyl sterols (31mg; 0.33μCi) were then isolated from the non-saponifiable lipid. When these sterols were subjected to t.l.c. on $AgNO_3$-impregnated silica gel with radioscanning and radioautography, no radioactivity was detected corresponding to cholesterol.

Incubations with [4-^{14}C]cholesterol and [4-^{14}C]-sitosterol. One *E. esculentus* was injected with 5μCi of [4-^{14}C]cholesterol and another animal with 2μCi of [4-^{14}C]sitosterol, and then the sea urchins were incubated for 2 and 6 days respectively. The 4-demethyl sterols were extracted in the usual manner and examined by t.l.c. on $AgNO_3$-impregnated silica gel. Radioautography showed that in both cases radioactivity only chromatographed with the Δ^5 monosaturated sterols. The sterols from the incubation with [4-^{14}C]sitosterol were further examined by g.l.c. (3% OV-17) and the effluent was trapped at 1min intervals and assayed for radioactivity. The radioactivity only chromatographed with a peak of the same retention time as sitosterol. Apparently dealkylation to cholesterol or other C_{27} or C_{28} sterol had not occurred.

Results

4-Demethyl sterols of the echinoid *Echinus esculentus* were shown to be predominantly Δ^5 sterols by testing with the Liebermann–Burchard reagent. Further examination by g.l.c. and g.l.c.–mass spectrometry of the trimethylsilyl ethers demonstrated that cholesterol was the major component (78.5%). Other constituents were identified as 26,27-dinorergosta-5,22-dien-3β-ol, cholesta-5,22-dien-3β-ol, cholesta-5,24-dien-3β-ol(desmosterol), 24-methylcholesta-5,22-dien-3β-ol, 24-ethylcholesta-5,22-dien-3β-ol, 24-methylcholest-5-en-3β-ol, 24-ethylcholest-5-en-3β-ol, 24-methylenecholest-5-en-3β-ol and 24-ethylidenecholest-5-en-3β-ol (Table 1). The absolute stereochemistry at C-24 of the 24-alkylated sterols was not determined.

The possible biosynthetic origins of these sterols, especially cholesterol, were investigated. When specimens of *E. esculentus* were incubated with [2-^{14}C]mevalonic acid for several days, radioactivity was efficiently incorporated into the non-saponifiable lipids (Fig. 1 and Table 2). A large proportion of the radioactivity corresponded to the 4-demethyl sterols (>30%). This is a complete contrast with the results obtained in similar experiments with starfish (Smith & Goad, 1971*b*). The remainder of the radioactivity was distributed between squalene (10–15%), 4,4-dimethyl sterols (10–20%), 4-monomethyl sterols (10–20%) and also polyprenols and ubiquinone (Walton & Pennock, 1972). For proof of synthesis, the squalene fractions were diluted with authentic material, purified through the formation of the squalene–thiourea adduct, and finally converted into the hexahydrochloride, which was then crystallized. In both experiments the radioactivity was retained throughout these procedures (Table 3). T.l.c. on $AgNO_3$-impregnated silica gel of the radioactive 4,4-dimethyl sterols as their acetates revealed that lanosterol was a major component (approx. 25% of the radioactivity) but that lanost-8-en-3β-ol was unlabelled. Further proof of the labelling of lanosterol was obtained by crystallization of the appropriate acetate samples with carrier compound to constant specific radioactivities. However, there were large initial decreases, presumed to be due to contamination by labelled Δ^{24}, 4,4-dimethyl-14-demethyl sterols, which were the most radioactive components of the 4,4-dimethyl sterols.

When a portion of the labelled 4-demethyl sterols from Expt. A was crystallized with added cholesterol a constant specific radioactivity was eventually obtained, but only after a large initial decrease. Initial purification of the labelled sterols by bromination followed by regeneration of the Δ^5 sterols gave a substantial decrease in specific radioactivity, indicating that much of the radioactivity was not present in Δ^5 sterol. T.l.c. on $AgNO_3$-impregnated silica gel of the sterols from both experiments revealed that only a small amount of radioactivity had an R_F value corresponding exactly to that of cholesterol but that a more heavily labelled compound had a similar R_F. When the cholesterol bands were removed and crystallized with authentic sterol, constant specific radioactivities were not attained until after large decreases in specific radioactivity occurred, presumably owing to the presence of an unidentified component, which could possibly be 5α-cholesta-7,24-dien-3β-ol, a known cholesterol precursor in the rat.

The other compounds seen on t.l.c. on $AgNO_3$-impregnated silica gel were more polar than cholesterol. One band had an R_F value corresponding to desmosterol, and when crystallized with authentic sterol the specific radioactivity rapidly became constant.

A specimen of *E. esculentus* was incubated with [26-^{14}C]desmosterol in order to observe if any saturation of the Δ^{24} bond could occur. However, t.l.c. of the 4-demethyl sterols on $AgNO_3$-impregnated silica gel did not provide any evidence for the formation of cholesterol. When another specimen of the sea urchin was incubated with 5α-[2-$^3H_2$]lanost-8-en-3β-ol and then the cholesterol purified either by

t.l.c. on $AgNO_3$-impregnated silica gel or through the formation of the 5,6-dibromide, specific radioactivities rapidly became constant on crystallization. Conversion of the cholesterol into 5α-6α-epoxycholestan-3β-ol followed by crystallization without any loss of radioactivity also confirmed the production of labelled cholesterol.

To test whether *E. esculentus* can demethylate 24-alkylated sterols, an animal was incubated with [4-^{14}C]sitosterol and then the sterols were isolated. G.l.c. of the sterols and monitoring the effluent for radioactivity showed that all recovered radioactivity corresponded to the injected compound, with none chromatographing with cholesterol. Also no further metabolism of injected [4-^{14}C]cholesterol to any other non-saponifiable lipid could be found even after several days of incubation.

Discussion

The composition of the 4-demethyl sterols of *E. esculentus* is in accordance with those reported (Bergmann, 1962; Gupta & Scheuer, 1968) for other species of echinoids. Cholesterol is the major component and it is accompanied by a variety of C_{26}, C_{27}, C_{28} and C_{29} Δ^5 sterols possessing both saturated and unsaturated side chains. The presence of 26,27-dinorergosta-5,22-dien-3β-ol and other C_{26} sterols in marine organisms has been reported previously (Idler *et al.*, 1970; Teshima *et al.*, 1972; Alcaide *et al.*, 1971; Viala *et al.*, 1972; Kobayashi *et al.*, 1972; Smith *et al.*, 1973; Goad *et al.*, 1972), but as yet their biosynthetic origins have not been established.

Our findings that *E. esculentus* is able to synthesize various labelled sterol precursors, including squalene, lanosterol and desmosterol, from [2-^{14}C]mevalonic acid is in apparent contrast with the results obtained by Salaque *et al.* (1966), who were unable to demonstrate any conversion of [1,2-^{14}C]acetate into squalene or sterols by another species of sea urchin, *Paracentrotus lividus*. These apparently conflicting results are probably not an effect of the use of different substrates or different species of echinoid, since Voogt (1972) has now demonstrated synthesis of squalene and sterol from acetate by using *P. lividus*. However, in this latter study the identity of the radioactive compounds detected in the sterols was not investigated. In *E. esculentus* we have now shown that though [2-^{14}C]mevalonic acid is converted into 4-demethyl sterols, very little of the radioactivity is in cholesterol, but desmosterol and possibly cholesta-7,24-dien-3β-ol are quite extensively labelled. The small amount of synthesis of cholesterol indicates perhaps that Δ^{24} sterol reductase activity is severely restricted in this organism under the conditions of the present experiments. This is supported by our failure to demonstrate any conversion of [26-^{14}C]desmosterol into cholesterol, yet the sea urchin is apparently quite able to metabolize 5α-[2-$^3H_2$]lanost-8-en-3β-ol to cholesterol. Also desmosterol has been detected in the sterol mixture of this echinoid. The Δ^{24} sterol reductase may perhaps act as a regulating enzyme in cholesterol biosynthesis in echinoids, dietary cholesterol possibly initiating a feedback mechanism.

No evidence was seen for any conversion of [2-^{14}C]mevalonic acid into any of the C_{26}, C_{28}, or C_{29} sterols present in *E. esculentus*, and they are presumed to be totally of dietary origin. Some insects are known to dealkylate phytosterols at C-24 to obtain cholesterol (Clayton, 1964) and the possibility exists that this mechanism also occurs in echinoids. However, [4-^{14}C]sitosterol is not dealkylated by *E. esculentus* in the present experiments and it seems unlikely that cholesterol is obtained in this manner.

In another class of echinoderms, the Asteroidea, cholesterol is converted into 5α-cholestan-3β-ol and 5α-cholest-7-en-3β-ol (Fagerlund & Idler, 1960; Smith & Goad, 1971*a*). However, we could detect no metabolism of [4-^{14}C]cholesterol into these compounds by *E. esculentus*.

The results reported here show that although *E. esculentus* can synthesize precursors of cholesterol rapidly from mevalonic acid, this is not translated appreciably into cholesterol synthesis. This may be due to the influence of the dietary cholesterol intake or may perhaps reflect seasonal variations in the rate of sterol synthesis. Both of the present experiments were carried out during the autumn, and it has been shown (Rubinstein, 1973) that in the sea urchin *Psammechinus miliaris*, sterol synthesis is maximal in June and July and minimal during the winter months.

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