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ON THE BIOSYNTHESIS OF 3β-STEROLS IN SOME REPRESENTATIVES OF THE ECHINOIDEA

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Abstract—1. The incorporation of sodium acetate-1-^{14}C into some classes of lipids in *Paracentrotus lividus*, *Echinus acutus* and *Psammechinus miliaris* is investigated.

2. It is demonstrated that these animals utilize the injected acetate for the biosynthesis of fatty acids and non-saponifiable lipids including squalene and 3β-sterols.

3. It is supposed that probably all echinoids are able to synthesize 3β-sterols.

INTRODUCTION

As a result of the use of radioisotopes our knowledge about the occurrence and distribution of the capacity of synthesizing 3β-sterols from lower units within the various phyla of the invertebrates has increased quickly in the past decade. Data about the sterol biosynthesis in the phylum Echinodermata have become available relatively late. Apart from the observation of Fagerlund & Idler (1960) that the starfish *Pisaster ochraceus* is able to transform the Δ-5 double bond of cholesterol to the C-7 position, and from that of Allen & Giese (1966) that the same species incorporates labeled acetate into the unsaponifiable lipids, more direct evidence about sterol biosynthesis in echinoderms was not obtained until 1966 when Salaque *et al.* injected sodium acetate-1,2-^{14}C into the echinoid *Paracentrotus lividus*. From the results of this experiment they concluded that, under their experimental conditions, *P. lividus* did not synthesize sterols or squalene from the precursor injected.

At that time data available suggested that the echinoderms could supply their need of sterols by converting the Δ^5-sterols obtained from the predated prey (mostly bivalves) into Δ^7-sterols. This was expressed by Fagerlund (1969) as follows:

"On the basis of the observed efficiency of this conversion ($\Delta^5 \rightarrow \Delta^7$, the author) and because the sterols of starfish are mostly C-7 unsaturated, while the major portion of the sterols of ingested mollusks are C-5 unsaturated, it may be assumed that the starfish converts not only 24-methylene cholesterol but the bulk of exogenous Δ^5-sterols to the corresponding Δ^7-sterols".

However, data did not exclude the possibility of sterolsynthesis by echinoderms, so that they might cover their need of sterols on the one side by conversion of the exogenous sterols offered, and on the other side by a synthesis *de novo*. For this reason it was decided in 1967 to investigate the occurrence and distribution of the capacity of synthesizing 3β-sterols within the phylum of the Echinodermata. This paper is the first one of a series describing the results of this investigation.

During this study Nomura *et al.* (1969) reported that the holothuroid *Stichopus japonicus* was able to synthesize squalene from acetate but that it was unable to cyclize this squalene to sterols. Smith & Goad (1971a) reported that the starfishes *Asterias rubens* and *Henricia sanguinolenta* did synthesize squalene, lanosterol, and to a lesser extent, cholest-7-enol from mevalonate though evidence was less clear in the case of *H. sanguinolenta*. In a following paper (Smith & Goad, 1971b) they reported that *A. rubens* and *Solaster papposus* can convert cholest-5-enol into cholest-7-enol and that 5α-cholestanol may be an intermediate in this conversion.

The foregoing shows that the sole positive indication for the presence of the capacity of synthesizing sterols within the phylum Echinodermata is concerned with the class Asteroidea. This paper deals with the biosynthesis of 3β-sterols in the echinoids *P. lividus*, *Echinus acutus* and *Psammechinus miliaris*.

MATERIALS AND METHODS

Specimens of *Paracentrotus lividus* were collected behind the laboratory for marine biology "Laboratoire Arago" at Banyuls-sur-mer, France, while those of *Echinus acutus* were obtained from the fishermen allied to that laboratory. Specimens of *Psammechinus miliaris* were obtained from the Netherlands Institute for Sea Research at Den Helder.

The animals were each injected with an aqueous solution of sodium acetate-1-^{14}C (Philips Duphar, sp. act. 20 mc/mM). All injections were given via the madreporite into the general body cavity. Data about the experimental animals, dosage and specific activity of the acetate injected are summarized in Table 1.

TABLE 1—DATA ABOUT THE ORIGIN AND TREATMENT OF *P. lividus*, *E. acutus* AND *P. miliaris* EXAMINED FOR THEIR CAPACITY OF SYNTHESIZING STEROLS

	P. lividus	*E. acutus*	*P. miliaris*
No. of animals	30	9	23
Place and data of collecting	Banyuls-sur-mer, 13 June, 1967	Banyuls-sur-mer, 15 June, 1967	N.I.S.R., 25 November 1966
Dosage administered to each animal (μc)	5	15	12·5
"Incubation time" (hr)*	87	72	121

* Time between the moment of injection and sacrificing the animals.

After the injection the animals were maintained in well aerated sea water. At the end of the incubation the specimens of *Paracentrotus* and *Echinus* were fixed in ethanol, while those of *Psammechinus* were frozen at −20°C.

Lipids were extracted from the animals using the modification of the method of Bligh & Dyer (1959) according to Van der Horst *et al.* (1969). The lipid extracts thus obtained

were washed as described by Folch (1957). Because in all cases strong emulsification occurred, lipids were subsequently purified, this is deprived of non-lipid contaminants by means of Sephadex G-25 (Terner *et al.*, 1970). Purified lipids made up about 97 per cent of the "total lipids". These purified lipids were hydrolysed with 1·5 N potassium hydroxide in 80% methanol for 6 hr under the usual conditions. The non-saponifiable and saponifiable lipids were isolated from the saponification mixture in the usual way. Thin-layer chromatography of the non-saponifiable lipids showed that these lipids were heavily contaminated with fatty acids in the case of *Paracentrotus* and *Psammechinus*, and weakly in the case of *Echinus*. These fatty acids were removed from the non-saponifiable lipids using the procedure after Capella *et al.* (1960) and then combined with the saponifiable lipids obtained directly from the saponification mixtures. The non-saponifiable lipids were separated into a crude squalene fraction, a crude sterol fraction and a remaining fraction by means of chromatography on columns of aluminium oxide (Merck) as described previously (Voogt, 1971a, b). Some carrier squalene was added to the crude squalene fraction and the squalenedodecabromide was prepared according to Mackenna *et al.* (1950). After several recrystallizations from ethylacetate a perfect white material was obtained. The 3β-sterols were obtained from the crude sterol fraction via their digitonides. Further purification of the sterols was performed by recrystallizing them from methanol.

Radioactivities were determined in toluene containing 4 g Omnifluor (NEN Chemicals) per l. in a Packard Tri-Carb, Type 3320 or 2420.

RESULTS

From *Paracentrotus* 14·7724 g of total lipids were obtained. Only a part of these, namely 2·9173 g, has been used, so that the amounts of all fractions obtained subsequently should be multiplied by the factor 5·063.

The quantities of the isolated lipid fractions are given in Table 2.

Samples were taken from all isolated lipid fractions to determine the specific and total radioactivity of the various fractions. The total radioactivities were also expressed as a percentage of the total amount of radioactivity administered to the animals and as a percentage of the radioactivity incorporated into the total lipids. These data are summarized in Table 3.

DISCUSSION

Table 2 shows great differences between the lipid contents of the three echinoids when they are expressed as a percentage of the fresh weight. It is likely these are, for the greater part, due to differences in the water content of the three species. This view is supported by the striking similarity of the lipid compositions; non-saponifiable lipids and 3β-sterols making up about 14·5 and 9 per cent of the total lipids respectively. When the amounts of 3β-sterols are expressed as a percentage of the fresh weight, the respective values of 0·06, 0·01 and 0·04 per cent are obtained. These values are low compared with those encountered in mollusks, the corresponding value there being about 0·08 per cent (Voogt, 1970, 1972).

Table 3 shows that all three species did synthesize lipids from the acetate injected, incorporation of radioactivity into lipids ranging between 2 and 4 per cent of the dosage administered.

In agreement with what could be expected the greater part of the activity incorporated into the lipids was found in the saponifiable fraction, but the

TABLE 2—QUANTITIES AND RELATIVE WEIGHTS OF ISOLATED LIPID FRACTIONS FROM *P. lividus*, *E. acutus* AND *P. miliaris*

Lipid fraction	*P. lividus*		*E. acutus*		*P. miliaris*	
	mg	Percentage of total lipids	mg	Percentage of total lipids	mg	Percentage of total lipids
Total fresh weight	2,200,000		2,730,000		226,000	
Total lipids	14,772·4		3372		1173·4	
(Percentage of fresh weight)	(0·67)		(0·12)		(0·52)	
Amount of total lipids used	2917·3		3372		1173·4	
Purified lipids	2840·9	96·3	3291·4	97·8	1146·4	97·9
Saponifiable lipids	2039·7	69·4	2513·5	74·9	879·4	76·4
Non-saponifiable lipids	430·2	14·7	476·0	14·3	164·8	14·7
Crude squalene fraction	12·6	0·4	25·2	0·8	9·6	0·9
Crude sterol fraction	343·1	11·8	371·6	11·2	106·7	9·8
3β-sterols	258·5	9·0	300·1	9·2	93·7	9·1

TABLE 3—RADIOACTIVITY OF THE ISOLATED LIPID FRACTIONS FROM *P. lividus*, *E. acutus* AND *P. miliaris* AFTER ADMINISTRATION OF SODIUM ACETATE-1-^{14}C EXPRESSED IN dpm/mg AND AS A PERCENTAGE OF THE RADIOACTIVITY INCORPORATED INTO THE TOTAL LIPIDS

Lipid fraction	*P. lividus*		*E. acutus*		*P. miliaris*	
	dpm/mg	Percentage of radioactivity in total lipids	dpm/mg	Percentage of radioactivity in total lipids	dpm/mg	Percentage of radioactivity in total lipids
Total dosage of radioactivity administered	$3{\cdot}33 \times 10^8$*		$2{\cdot}86 \times 10^8$*		$6{\cdot}3825 \times 10^8$*	
Total lipids (Percentage of total dosage)	516 (2·28)		3182 (3·75)		15,674 (2·88)	
Purified lipids	511·3	99·1	3380	100	15,710	98·2
Saponifiable lipids	623	86·2	3869	88·1	17,599	81·2
Non-saponifiable lipids	100	3·0	1107·6	4·8	6064·6	5·7
Crude squalene fraction	17		596		730·2	
Crude sterol fraction	74	1·8	936	3·2	3973·9	2·5
3β-sterols	56	1·0	490	1·4	2718	1·6
3β-sterols after three recrystallizations	48		207		1684	
3β-sterols after four recrystallizations	59		167		1646	
3β-sterols after five recrystallizations			184·4		1659	

* Expressed in dpm.

non-saponifiable lipids were also distinctly labelled. All fractions obtained after separation of the non-saponifiable lipids into a crude squalene fraction, a crude sterol fraction and a remaining fraction turned out to be radioactive. To the crude squalene fractions some carrier squalene was added. Because the amount of native squalene in these fractions is unknown, no calculations can be made about the diluting effect of this addition on the native squalene. The squalenedodecabromides were prepared and after several recrystallizations from ethylacetate the specific radioactivities amounted to 9 dpm/mg, 214 dpm/mg and 12 dpm/mg for *Paracentrotus*, *Echinus* and *Psammechinus*, respectively. After correcting these values for the influence of the bromine the corresponding specific radioactivities of the squalene were 30 dpm/mg, 706 dpm/mg and 40 dpm/mg, respectively. Because the dilutions, due to the addition of carrier squalene, are unknown we can only conclude from the foregoing that the specific radioactivities of the native squalene must be higher than the values calculated above. This means that radioactive acetate has been utilized for the biosynthesis of squalene.

The specific radioactivities of the 3β-sterols reached constant values after four or five recrystallizations from methanol and permit the conclusion that all three species did synthesize 3β-sterols from the acetate administered. The way of living and feeding of the echinoids under study (*Paracentrotus* being omnivorous, *Echinus* tending more to carnivorous) may be considered to be representative for the other members of this class of echinoderms. For this reason it seems probable that all representatives of the class Echinoidea are capable of synthesizing 3β-sterols. The results of this study are quite different from that of Salaque *et al.* (1966) which was also concerned with *P. lividus*. In that study sterols, which were not isolated via their digitonides but by means of column-chromatography, possessed a specific radioactivity of about 60 dpm/mg after one recrystallization from methanol. In the subsequent recrystallizations this radioactivity decreased very strongly and did not reach a constant value. Unfortunately, there seems to be no reasonable explanation for the different behaviour during recrystallization of the sterols in that and our study.

The identity of the sterols has been determined and will be dealt with in a separate paper.

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