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DEPOT STEROLS IN COMPARISON WITH STRUCTURAL STEROLS IN CANCER PAGURUS AND ERIOCHEIR SINENSIS

by

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CONTENTS

I. INTRODUCTION

All studies show that cholesterol is the predominant sterol of all crustacean species investigated. From the results of earlier publications it was shown that marine and fresh water crustaceans lack the ability to synthesize cholesterol from acetate or mevalonate (ZANDEE, 1962, 1964, 1966, 1967; VAN DEN OORD, 1964; GOSSELIN, 1965; O'CONNELL WHITNEY, 1969; TESHIMA & KANAZAWA, 1971b, 1971c). It was suspected that cholesterol and also minor sterols are of exogenous origin. The differences in the minor sterols may be caused by the diets.

In the 6 species studied by BODEA & CIURDARU (1968) cholesterol seems to be the only sterol. The sterol compositions of 14 species of Crustacea were determined by IDLER & WISEMAN (1971). Only in the spiny lobster *Panulirus argus* they found cholesterol as the sole sterol. In species of Euphausiacea and Decapoda (Macrura, Anomura and Brachyura), they found cholesterol as the major component. Desmosterol, when present, was the second principal sterol. Six other minor sterols were identified in certain species such as 22-dehydrocholesterol, brassicasterol, 22:23-dihydrobrassicasterol, 24-methylenecholesterol, β-sitosterol and 28-isofucosterol. O'CONNELL WHITNEY (1967) investigated the sterol composition of *Callinectes sapidus* and found cholesterol with small amounts of campesterol, β-sitosterol, stigmasterol and cholestanol. In *Balanus glandula* and *Balanus nubilis* a high percentage (*c.* 35%) of desmosterol was found by FAGERLUND & IDLER (1957) and O'CONNELL WHITNEY (1967), respectively.

KRITCHEVSKEY *et al.* (1967) found in a crab 22-dehydrocholesterol (3.9%), cholesterol (57.4%), brassicasterol (36.7%) and 24-methylenecholesterol (2.0%). Also the study of TESHIMA & KANAZAWA (1971c) on marine crustaceans show cholesterol as the major sterol (73 to 100%). In the lobster *Panulirus japonica*, the crab *Portunus trituberculatus*, and 3 species of mantis crabs, *Gonodactylus chiragra*, *Gonodactylus falcatus* and *Odontodactylus scayllarus* cholesterol was the sole sterol. In the prawn *Penaeus japonicus*, the amphipod *Caprella* sp. and the mysid *Neomysis intermedia* other sterols as 22-dehydrocholesterol and 24-methylenecholesterol were present. Brassicasterol was found in *Neomysis*, desmosterol in *Caprella* and trace amounts of β-sitosterol in *Penaeus* and *Neomysis*.

IDLER & WISEMAN (1971) isolated the sterols from decapod muscles and eggs and from euphausid whole bodies. O'CONNELL WHITNEY (1967) studied the sterol composition of the hepatopancreas (better is to speak of midgut gland; *cf.* VAN WEEL, 1974) and eggs of *Callinectes sapidus* while TESHIMA & KANAZAWA (1971a) studied sterol compositions isolated from entire animals. In all cases no separation was made in storage (midgut gland and ovaries inclusive eggs) and structural lipids (mainly from muscles and exoskeleton or animal without midgut gland and gonads). In all investigations mentioned so far no special attention was paid to the difference between the sterol composition of the midgut gland, in which mainly the depot sterols are present, and the remaining parts, which contain predominantly structural lipids, including sterols.

The early results of the investigations of RENAUD (1949) suggested that the crab *Cancer pagurus* puts sterols into store during the intermoult stage. Since it is known that several crustaceans are able to convert dietary sterols, especially the so-called "phytosterols" brassicasterol, ergosterol and β-sitosterol into cholesterol (TESHIMA, 1971a, 1971b; TESHIMA & KANAZAWA, 1971c, 1971d, 1972a, 1972b) it is worth looking at the possible difference between the composition of depot sterols and structural sterols isolated from the midgut gland and remaining parts.

The present paper deals with the sterol compositions of the different tissues of male and female specimens of the marine crab *Cancer pagurus* and of non-sexual mature, in fresh water living, specimens of the woolly handed crab *Eriocheir sinensis*.

II. MATERIALS AND METHODS

Specimens of the marine crab *Cancer pagurus* (L.) were obtained from the Netherlands Institute for Sea Research (N.I.O.Z.) at Texel while

specimens of *Eriocheir sinensis* (H. Milne Edwards) were collected from the fresh water lake Zuidlaardermeer in the vicinity of Groningen, The Netherlands.

The experimental animals which were in the intermoult phase, were kept in aquaria for a few days and fed with beef heart. From each animal the midgut gland and from the female *Cancer* also the deposited eggs with some surrounding tissue were separated. The different fractions were extracted in order to obtain the lipids by using the method described by BLIGH & DYER (1959). The total lipids were saponified in a solution of 1 N KOH in 80% methanol under the usual conditions.

The saponification mixture was diluted with same volume of water and the unsaponifiable lipids were extracted from the mixture with petroleum ether (b.p. 40 to 60° C). The petroleum ether was washed with water and the lipids obtained from the petroleum ether fraction were dried in a desiccator (VOOGT, 1971). The unsaponifiable lipids were separated into fractions by means of chromatography on columns of aluminium oxide (Woelm) such as described by VOOGT (1971). The isolated 3β-sterols were separated and identified by gas-liquid chromatography. A Becker gaschromatograph, Model 420 with dual columns and flame ionisation detection was used. The glass columns were 160 cm long and 0.38 cm inner diameter. Chromatography was performed as has been described by VOOGT (1971). Column temperatures were 215° C (SE-30) and 200° C (NPGS), respectively, while the temperature of the injection points was about 10° C higher.

The gasflow of both columns was about 40 ml N_2/min. Chromatography was carried out with sterols after recrystallization from methanol on SE-30 and with the trimethylsilylethers of the sterols on NPGS. Sterols were identified by comparing their steroid numbers, obtained on 2 different stationary phases, with those of reference sterols. Cholestane was used as an internal standard for determining steroid numbers with the formula given by VOOGT (1971). As a check on the identification, sterols were also hydrogenated (VOOGT, 1971).

III. RESULTS

The quantities of the isolated lipid fractions are given in Table I for *Cancer pagurus* and in Table II for *Eriocheir sinensis*. The results of the gas-liquid chromatographical analyses and the proportional composition of the sterol mixtures are given in Table III for both *Cancer pagurus* and *Eriocheir sinensis*. Representative chromatograms of the remaining parts of the male *Eriocheir sinensis* are shown in Fig. 1 on the stationary phases SE-30 and NPGS, respectively.

TABLE I

Quantities of the isolated lipid fractions from *Cancer pagurus*; one animal for each sex.

	Remaining parts		*Midgut gland*		*Eggs*
	Male	*Female*	*Male*	*Female*	
Total fresh weight (g)	385	630	25	32	45
Total lipids (mg)	2068	2778	4286	4693	2578
% *of fresh weight*	0.5	0.4	17.1	14.7	5.7
Unsaponifiable lipids (mg)	132	189	97	83	145
% *of fresh weight*	0.03	0.03	0.39	0.26	0.32
% *of total lipids*	6.4	6.8	2.3	1.8	5.6
Crude sterol fraction (mg)	79	99	63	45	62
% *of fresh weight*	0.02	0.02	0.25	0.14	0.14
% *of total lipids*	3.8	3.6	1.5	1.0	2.4

TABLE II

Quantities of the isolated lipid fractions from *Eriocheir sinensis*.

	Remaining parts		*Midgut gland*	
	Male	*Female*	*Male*	*Female*
Number of animals	4	4	4	4
Total fresh weight (g)	650	455	60	46
Total lipids (mg)	7862	6678	24108	17480
% *of fresh weight*	1.2	1.5	40.2	38.0
Unsaponifiable lipids (mg)	549	456	330	295
% *of fresh weight*	0.08	0.10	0.55	0.64
% *of total lipids*	7.0	6.8	1.4	1.7
Crude sterol fraction (mg)	319	245	115	92
% *of fresh weight*	0.05	0.05	0.19	0.20
% *of total lipids*	4.1	3.7	0.5	0.5

IV. DISCUSSION

From the results of Table I it is clear that in *Cancer pagurus* there is a big difference in lipid content between the midgut gland and the animal without midgut gland (remaining parts). The lipids of the remaining parts represent for the greater part structural lipids. In the male animal the quantity of lipids amounts to 0.5% and in the female to 0.4% of the fresh weight. In the midgut gland the amounts are 17.1% and 14.7%, respectively. The lower quantity of lipids in the female midgut gland will be due to the storage of lipids in the ovaries and eggs. The same phenomenon is shown for the unsaponifiable lipids and the crude sterol fraction of the male midgut gland in comparison with the female one and the eggs. The quantities of the unsaponifiable

TABLE III

Proportional composition of the sterol mixtures of *Cancer pagurus* and *Eriocheir sinensis*.

Sterol	*Cancer pagurus*					*Eriocheir sinensis*			
	Midgut gland		*Eggs*	*Remaining parts*		*Midgut gland*		*Remaining parts*	
	Male	*Female*		*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
22-dehydrocholesterol	4.2	4.8	3.7	2.8	3.1	1.5	2.0	1.3	1.6
cholesterol	88.1	89.5	92.1	90.8	93.0	87.2	82.6	90.5	89.1
brassicasterol	2.9	1.9	0.7	2.2	1.4	1.9	2.3	2.3	2.8
campesterol	3.7	2.4	2.2	2.9	1.5	4.3	5.7	2.8	2.8
stigmasterol	0.3	0.8	0.8	0.6	0.6	0.4	0.9	0.4	0.5
β-sitosterol	0.6	0.5	0.5	0.5	0.4	4.7	6.5	2.7	3.2
	0.2	trace	0.0	0.2	0.0	0.0	0.0	0.0	0.0

lipids of the remaining parts in both the male and female animal are equal and very low, *viz.* 0.03% of the fresh weight, whereas the crude sterols amount 0.02% of the fresh weight.

The comparable results of *Eriocheir sinensis* which are shown in Table II demonstrate a much higher quantity of lipids (as percentage of the fresh weight) in both the midgut gland and remaining parts of males and females. The percentages of the crude sterols are 0.2% (midgut gland) and 0.05% (remaining parts) of the fresh weight; there are no differences between the male and female animals, because they were not in a reproductive phase.

The proportional composition of the sterol mixtures of *Cancer pagurus* and *Eriocheir sinensis* is given in Table III. In both the animals cholesterol is the main sterol. The differences in cholesterol content between the male and female fractions (midgut gland or remaining parts) are very low except for the *Eriocheir* midgut gland. The differences between the midgut gland and remaining parts fractions of the males and females are more pronounced. In *Cancer pagurus* the β-sitosterol content is much lower than in *Eriocheir sinensis* and causes the higher cholesterol content in *Cancer pagurus* in comparison with *Eriocheir sinensis*. In both the species investigated we were unable to demonstrate desmosterol.

It seems that there is a preference for cholesterol in structural lipids. The question is whether all sterols found can have a structural function in both animals. TESHIMA & KANAZAWA (1971a) found that in *Portunus trituberculatus* 100% of the sterols was composed by cholesterol. The same authors (1972b) found *in vivo* bioconversion of β-sitosterol to cholesterol in the same crab, and TESHIMA (1971b) has shown *in vivo* transformation of ergosterol to cholesterol. Until now these possibilities have not been shown for *Cancer pagurus* and *Eriocheir sinensis*. The presence of C_{28} and C_{29} -sterols in structural lipids could result from the

absence of bioconversion reactions in these animals; otherwise it would be possible that these sterols are no components of membrane structures, but that they are found in different organs as circulating sterols waiting for bioconversion.

In the female *Cancer* a higher percentage of cholesterol is present in

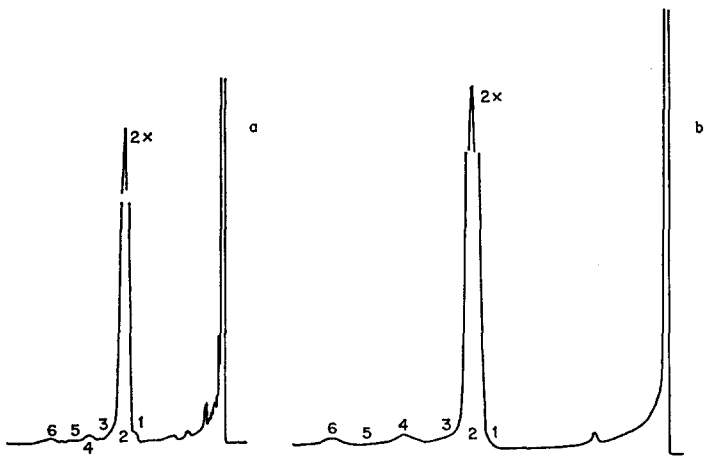

Fig. 1. Chromatograms of the sterols and of the trimethylsilyl derivatives of the sterols of the remaining parts of the males of *Eriocheir sinensis* after GLC separation on the stationary phases SE-30 and NPGS, respectively. a. Sterols; the percentage composition, the steroid numbers found, and the steroid numbers of the suitable sterols (in parentheses) are: 1) 1.3% 22-dehydrocholesterol 29.04; 2) 90.5% cholesterol 29.47 (29.42); 3) 2.3% brassicasterol 29.78 (29.85); 4) 2.8% campesterol 30.40 (30.42); 5) 0.4% stigmasterol 30.78 (30.75); 6) 2.7% β-sitosterol 31.30 (31.30). b. Trimethylsilyl derivatives; the percentage composition, the sterol numbers found, and the steroid number of the suitable sterols (in parentheses) are: 1) 0.8% 22-dehydrocholesterol 30.03 (30.09); 2) 94.8% cholesterol 30.38 (30.33); 3) 0.8% brassicasterol 30.76 (30.83); 4) 1.5% campesterol 31.41 (31.44); 5) 0.3% stigmasterol 31.70 (31.70); 6) 1.8% β-sitosterol 32.22 (32.28).

comparison with the male. The eggs show nearly the same cholesterol percentage (92.1) as in the female animal without midgut gland, whereas the sterol content is the same as in the midgut gland (Table II).

It is worthwhile to check the function of the minor sterols.

V. SUMMARY

The differences in sterol content and sterol composition between the midgut gland and remaining parts (structural lipids) of male and female specimens of *Cancer pagurus* and *Eriocheir sinensis* are investigated. There are no differences in sterol content in the structural lipids

between male and female specimens of the same species. Also the sterol content in the midgut gland of male and female *Eriocheir sinensis* is equal, whereas in the midgut gland of the male specimens of *Cancer pagurus* the sterol content is about twice as high as in the midgut gland of the reproductive female one.

The main sterol is cholesterol (82 to 93%) in both sexes of both species investigated. The highest cholesterol contents are found in the structural lipids of both specimens and also in the eggs of *Cancer pagurus*.

Small quantities of campesterol, brassicasterol, 22-dehydrocholesterol, β-sitosterol and stigmasterol are shown in all fractions.

The principal difference in sterol composition between the two species is the higher quantity of campesterol and β-sitosterol in *Eriocheir sinensis* in exchange for cholesterol in *Cancer pagurus*.

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