ABSENCE OF CHOLESTEROL SYNTHESIS AS CONTRASTED WITH THE PRESENCE OF FATTY ACID SYNTHESIS IN SOME ARTHROPODS

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Abstract—1. After administration of acetate-1-^{14}C absence of cholesterol synthesis was demonstrated in the lobster *Homarus gammarus* (L.), the spider *Avicularia avicularia* (L.) and in the millepede *Graphidostreptus tumuliporus* (Karsch).

2. However, the animals utilize acetate for the synthesis of fatty acids (except for polyunsaturated ones) and for some non-saponifiable lipids.

3. Gas–liquid chromatography analyses demonstrated even- and odd-numbered fatty acids, saturated and unsaturated ones, *iso*, *anteiso* and probably *neo*-branched-chain fatty acids.

4. The fatty-acid composition of the mixtures from the animals and the biosynthesis of the fatty acids are discussed.

INTRODUCTION

IT IS recognized that insects generally require a dietary sterol for optimum growth and development (Gilmour, 1961; Clayton, 1964). Many species of insects, mostly phytophagous and omnivorous ones, can modify the sterols from their diet (Bergmann, 1962).

Isotopic tracer techniques have been used to demonstrate the absence of cholesterol synthesis in insects (Bloch *et al.*, 1956; Clark & Bloch, 1959; Kodicek & Levinson, 1960; Robbins *et al.*, 1960; Sedee, 1961; Kaplanis *et al.*, 1961, 1963).

It is interesting that the insects are not the only arthropodean class in which the biosynthesis of sterols is absent or defective. Crustacea too fail to utilize acetate to synthesize cholesterol. This was found for the crayfish *Astacus astacus* (L.) (Zandee, 1962), studied for several seasons, after administration of sodium acetate-1-^{14}C (Zandee, 1964, 1966a) and after administration of mevalonic acid-2-^{14}C (Gosselin, 1965). The crab *Cancer pagurus* (L.) lacks the ability to synthesize cholesterol from acetate-1-^{14}C or mevalonic acid-2-^{14}C too (Van den Oord, 1964a).

The present study reports the absence of cholesterol biosynthesis in another crustacean species, namely *Homarus gammarus* (L.). One representative of the class Arachnida, *Avicularia avicularia* (L.), and one of the Myriapoda, *Graphidostreptus tumuliporus* (Karsch), have been studied in this order. Information on these Arthropoda classes would be of great interest since the results obtained with insects and crustaceans do suggest that the phenomenon will range over the whole phylum Arthropoda.

To investigate the phenomenon mentioned in these animals sodium acetate-1-^{14}C was used. Many investigators prefer an immediate precursor like mevalonic acid or squalene for the study of the biosynthesis of sterols in animals (Fagerlund & Idler, 1960; Wootton & Wright, 1962). When mevalonic acid or squalene is not incorporated into the cholesterol the results are evidence for the absence of sterol synthesis. However, there is no evidence at all of metabolic activity for other compounds like fatty acids or amino acids. It is possible that in animals like mussels, which are injected at random (Fagerlund & Idler, 1960), the dose will be lost in the surrounding water. Moreover, it may occur that metabolically the animals are barely active so that hardly any incorporation of ^{14}C of the acetate into the isolated substances will be found. It is also conceivable that the animals investigated did not synthesize sterols during the experiments, since a sufficient amount of that material was already present. This will especially apply to metabolites with a small turnover rate.

In this paper the fatty-acid composition of the animals investigated is described and the biosynthesis of the fatty acids is also discussed.

MATERIALS AND METHODS

The lobster, *Homarus gammarus*, was caught in the North Sea near the coast of Holland in summer. The animal was kept in running sea water at a temperature of 16°C.

Three spiders, *Avicularia avicularia*, were obtained from Equador via a banana importer. They were kept at a temperature of 25°C and fed with *Tenebrio* larvae.

Ten millepedes, *Graphidostreptus tumuliporus*, were collected from the vicinity of the Institut Français d'Afrique Noir, Dakar, Senegal. In the laboratory they were placed in glass troughs, containing moist soil, at a temperature of 26°C. They were fed with vegetables and fruit and were provided with water.

The lobster, fresh weight 1663 g, was injected four times at intervals of 24 hr. Each injection consisted of 100 μc of sodium acetate-1-^{14}C (specific activity, 10 μc/mg) in 1 ml of water. The three spiders (total fresh weight 20·6 g) were injected with the same acetate solution. They received one injection of about 1 μc/g animal. In the same way ten millepedes (total fresh weight 102 g) were injected with about 1 μc/g animal. After a period of 4 days the lobster was killed and divided into three portions, namely hepatopancreas, muscles and remaining parts. Each portion was saponified in 1·5 N potassium hydroxide in 80% methanol. The spiders and millepedes were killed 24 hr after injection and saponified whole in 1·5 N potassium hydroxide in 80% methanol. The saponifications were carried out under reflux for about 5 hr in an atmosphere of nitrogen. The saponification mixtures were then filtered and the non-saponifiable and the saponifiable lipids were extracted with light petroleum ether in the usual manner. The extracts were pooled and dried over anhydrous sodium sulphate. In addition the saponifiable lipid fraction (fatty acids) was treated with activated charcoal (B.D.H.). Further preparation of the non-saponifiable material to be used for isotope determinations was carried out by chromatography on columns of aluminium oxide as previously

described (Zandee, 1966a). Activities from the various fractions were determined on $BaCO_3$ samples of infinite thickness* or on infinitely thin layers† on aluminium planchets with a Philips Geiger–Müller counter.

The isolated fatty acids from the saponifiable lipid fraction were stored in petroleum ether at $-20°C$. The methyl esters of the fatty acids obtained by the method of Schlenk & Gellerman (1960) were analysed by gas–liquid chromatography. The analyses were made with a Becker instrument equipped with katharometer detection. Columns were filled with 20 per cent by weight of ethylene glycol adipate polyester and 2 per cent of phosphoric acid on 60–80 mesh Chromosorb-W. The column temperature was approximately 182°C. Further details and identification procedures have been described in an earlier report (Zandee, 1966b). Peaks of the original and the hydrogenated samples were collected on anthracene cartridges (according to Karmen *et al.*, 1962) and the radioactivity of the fatty acids was determined with an I.D.L. liquid scintillation counter, type 2022. The activities were expressed in counts.min^{-1} mg^{-1} methylated fatty acid (specific activity) at 100 per cent efficiency.

RESULTS

The quantities of the isolated lipid fractions are summarized in Table 1. The high content of unsaponifiable material, especially sterols, and also fatty acids in the hepatopancreas of *Homarus* as compared with the muscles and remaining parts, is in conformity with the known results for *Cancer pagurus* (Renaud, 1949; Vonk, 1960). It is remarkable that in all animals the quantity of the crude squalene fractions was greatly polluted with hydrocarbons and perhaps wax-like substances.

TABLE 1—QUANTITIES OF ISOLATED LIPID FRACTIONS

	Homarus				*Avicularia*	*Graphido-streptus*
	Hepato-pancreas	Muscles	Remaining parts	Total		
Total fresh wt. (mg)	70,000	369,000	1,220,000	1,659,000	20,600	102,000
Unsaponifiable lipids (mg)	668	173	260	1101	83	108
(Fresh wt., %)	0·95	0·05	0·02	0·07	0·40	0·11
Crude squalene (mg)	43	—	13	56	7	6
Crude sterols (mg)	380	138	141	659	48	86
(Fresh wt., %)	0·543	0·037	0·011	0·04	0·233	0·084
Fatty acids (mg)	2740	78	6430	9248	170	101
(Fresh wt., %)	3·91	0·02	0·53	0·56	0·83	0·10

* According to Calvin *et al.*, 1949.
† Counts were corrected to zero mass.

The radioactivity of the crude squalene fractions appeared to belong to the hydrocarbons only. The squalene hexahydrochlorides formed by the Heilbron method and modified by Loud & Bucher (1958) nowhere showed radioactivity. In the carnivorous spiders a higher percentage of sterols and fatty acids was found than in the herbivorous millepedes.

The results of the radioactivities of the isolated lipid fractions are shown in Table 2. Neither cholesterol nor squalene contains radioactivity, so it must be concluded that these representatives of the phylum Arthropoda cannot synthesize cholesterol or other sterols from acetate either. In all cases, however, the animals utilized acetate for the synthesis of fatty acids and some non-saponifiable lipids. The activity of the wax-like substances and hydrocarbons from both *Avicularia* and *Graphidostreptus* was notably high.

TABLE 2—RADIOACTIVITY* OF THE ISOLATED LIPID FRACTIONS EXPRESSED IN COUNTS/MIN
AFTER ADMINISTRATION OF ACETATE-1-^{14}C

| | *Homarus* | | | | |
	Hepato-pancreas	Muscles	Remaining parts	*Avicularia*	*Graphido-streptus*
Unsaponifiable fraction	$10\cdot8 \pm 0\cdot6$	—	$89\cdot1 \pm 1\cdot1$	$85\cdot3 \pm 1\cdot0$	$1712\cdot1 \pm 4\cdot1$
Wax-like substances from the unsaponifiable fraction	$11\cdot2 \pm 0\cdot4$	—	—	$552\cdot5 \pm 2\cdot3$	$10{,}975\cdot0 \pm 11\cdot0$
Squalene hexahydrochloride	$0\cdot5 \pm 0\cdot4\dagger$	—	$0\cdot3 \pm 0\cdot4\dagger$	$0\cdot4 \pm 0\cdot5\dagger$	$0\cdot9 \pm 0\cdot6\dagger$
Cholesterol digitonide	$0\cdot5 \pm 0\cdot8$	$0\cdot3 \pm 0\cdot5$	$0\cdot6 \pm 0\cdot5$	$0\cdot9 \pm 0\cdot6$	$0\cdot2 \pm 0\cdot5$
Cholesterol	$0\cdot06 \pm 0\cdot4$	$0\cdot1 \pm 0\cdot5\dagger$	$0\cdot1 \pm 0\cdot5$	$-0\cdot2 \pm 0\cdot5$	$1\cdot0 \pm 0\cdot6$
Fatty acids	$13\cdot1 \pm 0\cdot6$	$29\cdot4 \pm 0\cdot5$	$170\cdot5 \pm 1\cdot4$	$91\cdot1 \pm 1\cdot1$	$1292\cdot1 \pm 3\cdot6$
Melting point of cholesterol (°C)	$146\cdot7$	—	$147\cdot6$	$148\cdot0$	$146\cdot3$

* In counts/min per infinitely thick $BaCO_3$ sample measured with the G.–M. tube.
† Infinitely thin layers on aluminium planchets.

The identities and the percentage composition of the fatty acids are summarized in Table 3. The values presented in this table are the mean values of at least five chromatograms with at least two runs of hydrogenated mixtures. The fatty-acid analyses show large amounts of palmitic (16:0) and oleic (18:1) acid in all cases. There are important differences between the fatty-acid composition of the hepatopancreas, muscles and remaining parts of *Homarus*. The hepatopancreas showed the greater variety in fatty acids. Straight and branched-chain saturated acids with an even and odd number of carbon atoms were present. Mono-unsaturated acids of almost all straight-chain acids were found. It is remarkable that three types of eicosenic acids (20:1), with a quantity of 10·7 per cent and the continuation of it

TABLE 3—FATTY ACID COMPOSITION OF *Homarus*, *Avicularia* AND *Graphidostreptus*

Shorthand designation	Retention time relative to 18:0	Homarus			Avicularia (%)	Graphido-streptus (%)
		Hepato-pancreas (%)	Muscles (%)	Remaining parts (%)		
Up to 14:br.		1·7	3·2	1·7	3·5	1·0
14:0	0·260	1·6	1·0	2·1	2·5	0·7
14:1	0·310	—	—	—	0·3	—
15:0 *neo*	0·296	0·1	0·9	0·1	—	—
15:0 *iso*	0·310	0·4	0·8	0·5	0·2	0·6
15:0 *anteiso*	0·330	0·2	0·2	0·1	—	0·3
15:0	0·362	0·7	0·3	1·3	0·4	0·8
15:1	0·423	—	0·4	0·1	0·1	0·3
16:0 *neo*	0·370	0·2	4·0	0·2	—	—
16:0 *iso*	0·436	0·4	14·5	0·4	2·6	0·8
16:0 *anteiso*	0·463	0·2	1·0	—	—	—
16:0	0·517	16·9	16·0	25·7	31·0	25·6
16:1	0·586	10·3	6·0	10·5	2·2	3·7
17:0 *neo*	0·531	1·0	2·8	3·0	0·8	0·6
17:0 *iso*	0·602	2·2	2·3	2·2	0·8	0·4
17:0 *anteiso*	0·651	1·3	0·3	0·5	0·4	0·3
17:0	0·710	1·3	0·5	1·4	1·2	0·7
17:1	0·804	1·1	1·0	2·5	0·7	2·0
18:0 *neo*	0·720	0·4	2·5	Trace	—	0·3
18:0 *iso*	0·851	0·8	5·8	0·9	0·2	—
18:0 *anteiso*	0·892	0·3	1·0	Trace	—	—
18:0	1·00	2·4	7·7	6·8	12·6	5·4
18:1	1·12	29·9	20·7	34·2	36·0	42·5
18:2	1·35	0·1	Trace	0·1	0·2	2·0
18:3	1·73	Trace	—	—	—	—
19:0 *neo*	1·02	0·8	2·3	0·5	—	2·0
19:0 *iso*	1·16	0·7	Trace	—	0·6	—
19:0 *anteiso*	1·25	0·6	Trace	0·3	0·3	0·4
19:0	1·37	0·3	0·4	0·3	0·5	0·8
19:1	1·53	2·0	—	0·9	0·2	Trace
20:0 *neo*	1·37	—	0·6	—	—	0·9
20:0 *iso*	1·59	Trace	1·2	—	0·1	—
20:0 *anteiso*	1·8	—	—	—	—	0·1
20:0	1·89	0·7	0·5	0·6	1·2	1·4
20:1	2·11	4·8	2·1	2·1	0·7	1·0
20:1	2·16	4·2	0·4	>0·7	—	—
20:1	2·26	1·7	0·8		0·8	—
20:2	2·52	0·9	0·1	0·2	—	2·0
20:3	2·87	0·7	0·2	—	—	—
20:4	3·17	Trace	—	—	—	—
21:0 *neo*	2·08	—	—	—	—	0·6
21:0 *iso*	2·19	—	—	—	—	0·4
21:1 *neo* ?	2·41	—	—	—	—	1·2
21:0	2·60	0·2	—	—	—	—

TABLE 3—continued

Shorthand designation	Retention time relative to 18:0	Homarus			Avicularia (%)	Graphido-streptus (%)
		Hepato-pancreas (%)	Muscles (%)	Remaining parts (%)		
21:1	2·99	0·4	0·2	—	—	—
22:0	3·66	0·5	—	0·1	0·2	0·8
22:1	3·97	1·2	—	Trace	Trace	—
22:1	4·11	1·3	—	Trace	—	—
22:1	4·39	1·9	—	Trace	—	—
23:0 neo ?	4·08	—	—	—	—	0·3
24:0 neo ?	5·75	—	—	—	—	0·5
24:0	6·93	1·5	—	—	—	—
25:0 neo ?	7·49	1·1	—	—	—	—

in 22:1 acids with a quantity of 3·4 per cent were found in the hepatopancreas and to a lower extent in the muscles and remaining parts of *Homarus*.

Polyunsaturated acids were demonstrated for C_{18} and C_{20} acids only. On the strength of available markers and known retention times *iso* and *anteiso* fatty acids could be demonstrated. The *neo*-type was identified only on the ground of published retention times (Farquhar *et al.*, 1959) and is identified tentatively only. However, it is of interest that we found a homologue series for this possible type of branching. The muscles of *Homarus* contain a considerable amount of branched acids (40·6 per cent) compared to the hepatopancreas (9·6 per cent) and the remaining parts (8·7 per cent). Also in *Avicularia* and *Graphidostreptus* branched-chain fatty acids were detected. The *iso*-type proved to be predominant in *Avicularia* and the *neo*-type, provided that the provisional identification is correct, in *Graphidostreptus*. Polyunsaturated fatty acids were scarce in both *Avicularia* and *Graphidostreptus*.

Table 4 presents the radioactivities of the fatty acids or of the conglomerates of some of these acids. The radioactivity is expressed in counts.min^{-1} mg^{-1} methylated fatty acid or conglomerate of fatty acids, because there was no complete separation.

Table 5 gives the radioactivities of 1 mg of the total mixtures.

The values under column II of Table 5 are in good agreement with those obtained by direct counting of 1 mg methyl esters in PPO–POPOP–toluene* solution. Transesterifications (Pascaud, 1963) were checked. They were avoided by carrying out the analyses at about 182°C.

The lobster hepatopancreas shows little activity in the fatty acids compared with the fatty acids of both the muscle and remaining parts. This might be expected from an isotope dilution effect. On the whole the radioactivity of the even- and odd-numbered straight-chain fatty acids is found to be proportional to

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* PPO, 2,5-diphenyloxazole. POPOP, 1,4-bis[2-(5-phenyloxazolyl)]-benzene.

TABLE 4—THE DISTRIBUTION OF ^{14}C IN THE DIFFERENT FATTY ACIDS AFTER ADMINISTRATION OF ACETATE-1-^{14}C. RADIOACTIVITY IN COUNTS.MIN^{-1} MG^{-1} METHYLATED FATTY ACID (SPECIFIC ACTIVITY)

| Shorthand designation | Homarus | | Avicularia | Graphido-streptus |
	Hepato-pancreas	Remaining parts		
Up to 14:br.	129	3178	443	13,500
14:0	103	4329	1092	18,557
14:1	—	—	100	—
15:0 neo	150	} 1228	—	—
15:0 iso	} 183		2017*	} 10,800
15:0 anteiso			—	
15:0	} 59	561	—	4050
16:0 neo			—	—
16:0 iso	} 27	} 642	408	6800
16:0 anteiso			—	—
16:0	252	3471	1573	18,858
16:1	55	619	864	6621
17:0 neo			—..	
17:0 iso	} 191	} 650	925	} 5446
17:0 anteiso	270		—	
17:0	165	1278	1166	13,471
17:1	50	} 848	14	3615
18:0 neo	275		—	18,400
18:0 iso	} 172	} 1370	4800	—
18:0 anteiso			—	
18:0	1142	5736	2173	49,394
18:1	74	555	141	4353
18:2	—	—	—	—
18:3	—	—	—	—
19:0 neo			—	
19:0 iso	} 322	} 1100	1750	} 10,555†
19:0 anteiso	232		1666	
19:0	256‡	2833§	1800	52,100
19:1	670	2711	2050	?
20:0 neo	?	?	—	
20:0 iso	?	—	13,300	} 6333
20:0 anteiso	—	—	—	
20:0	1263	7633	3150	27,800
20:1			1071	12,875‖
20:1	} 135	} 4429	—	—
20:1			2475	—
20:2	66	2700	—	?
20:3	23	—	—	—
20:4	—	—	—	—
21:0 neo	—	—	—	?
21:0 iso	—	—	—	105,466¶
21:0	160	—	—	171,000
21:1 neo?	—	—	—	?

TABLE 4—*continued*

Shorthand designation	Homarus		Avicularia	Graphido-streptus
	Hepato-pancreas	Remaining parts		
21:1	?	—	—	—
22:0	2360	9285	21,550	118,925
22:1	⎫	⎫	?	—
22:1	⎬ 402	⎬ ?	—	—
22:1	⎭	⎭	—	—
23:0 *neo?*	—	—	—	⎫
24:0 *neo?*	—	—	—	⎬ 108,050
24:0	215	—	—	⎭
25:0 *neo?*	309	—	—	—

* Including 15:0.   † Except 19:0 *iso*.
‡ Including 20:0 *neo*.   § Including 20:0 *neo*.
‖ Except 20:0 *iso*.   ¶ Including 20:2, 21:1.

TABLE 5—THE RADIOACTIVITY OF 1 mg METHYLATED FATTY ACID MIXTURE

	I	II
Homarus		
Hepatopancreas	182·5	186·1
Muscle	395·3	—
Remaining parts	2048·0	2030·4
Avicularia	1184·0	1102·4
Graphidostreptus	18,201·1	18,163·9

I. The radioactivity of 1 mg methylated fatty acids, determined by liquid scintillation counting in PPO-POPOP, according to the method of Hayes *et al.* (1956). The results expressed in counts/min were calculated at 100 per cent counting efficiency.
II. The radioactivity in counts/min after summation of the radioactivities (determined on anthracene cartridges by scintillation counting) of the separate components from 1 mg mixture of methyl esters.

the increase of the number of carbon atoms. This phenomenon is also found in *Avicularia* and *Graphidostreptus*. The biosynthesis of these fatty acids must take place almost completely by way of the elongation system, by either the mitochondrial (Wakil, 1961, 1962) or microsomal system (Nugteren, 1965). Possibly the mono-unsaturated acids have been synthesized from the corresponding saturated acids, because the labelling with radiocarbon of the former is lower than the latter in all cases except that of 19:1, which exceeded that of 19:0. However, it is possible that during the experiments most of the produced 19:0 was dehydrogenated directly to 19:1.

The polyunsaturated acids, linoleic (18:2), linolenic (18:3) and arachidonic (20:4), nowhere show radioactivity. In *Astacus astacus* (Zandee, 1966b) linoleic acid was stated to be essential; it is possible that the same is true for *Homarus*, although in this animal very much less of this acid is present. The branched-chain fatty acids are labelled distinctly, and the labelling is relatively high in all the animals investigated.

DISCUSSION

The experiments described in this paper show that the representatives of the Arthropoda, namely *Homarus gammarus* (L.), *Avicularia avicularia* (L.) and *Graphidostreptus tumuliporus* (Karsch), do not incorporate acetate into cholesterol or into its precursor, squalene. These findings agree with the results found for the crustaceans *Astacus astacus* (L.) (Zandee, 1962, 1964, 1966a), *Cancer pagurus* (L.) (Van den Oord, 1964), *Lepas anatifera* (L.) and *Eupagurus bernhardus* (L.) (Zandee, unpublished data). The results found for insects, for crustaceans and for *Avicularia* and *Graphidostreptus* support the assumption that all the groups of Arthropoda have a dietary requirement for cholesterol or other sterols.

The predominant sterol, cholesterol, present in the animals investigated is of dietary origin. The carnivorous animals will find cholesterol in their food but a herbivorous animal like the diplopode *Graphidostreptus* will not. The latter depends on the phytosterols of its food. Since cholesterol is the most important sterol in *Graphidostreptus* it must be supposed that the animal can modify phytosterols into cholesterol. The amount of cholesterol in the animals of the phylum Arthropoda investigated is very small compared with that of the vertebrates. It is a known fact that most vertebrates produce large quantities of bile acids from cholesterol. The bile salts are highly important for lowering the surface tension and increasing the emulsification, the digestion and absorption of fat and fat-soluble vitamins.

In the gastric juice of Crustacea and of other invertebrates surface active compounds were shown to be present (Vonk, 1935, 1960, 1962). It was demonstrated by Van den Oord *et al.* (1964) that no bile acids or bile alcohols were present in the gastric juice of the crab *Cancer pagurus*. The isolated emulsifiers in the crab appeared to be fatty acylsarcosyltaurines (Van de Oord *et al.*, 1965). Recently the biosynthesis of the emulsifiers from the gastric juice of the crab *Cancer pagurus* has been described by Van den Oord (1966). It was demonstrated that no sterols are involved in emulsifying properties of the gastric juice. It is possible that no bile acids will be found in invertebrates and that inability to synthesize cholesterol is correlated with the inability to degrade cholesterol to bile acids.

The fatty-acid composition of *Homarus gammarus* is in agreement with that of *Astacus astacus* (Zandee, 1966b). The essential fatty acids (18:2 and 18:3), so called because no labelling was found in these acids, are present in *Astacus* in considerable amounts. The much lower percentages of the essential fatty acids and other polyunsaturated fatty acids in *Homarus* may be due to the season and to the temperature of the North Sea.

In *Astacus* of Polish origin 0·43 per cent, and in *Astacus* of Yugoslav origin 0·13 per cent of fatty acids (of the total fresh weight) were found (Zandee, 1966a). For linoleic acid we have demonstrated an average percentage of 10·1 per cent in the fatty-acid mixtures of Polish origin and of 3·7 per cent in those of Yugoslav origin (Zandee, 1964).

The fatty-acid mixtures of *Graphidostreptus* and *Avicularia* were liquid at 25–30°C just as the mixtures of *Astacus* and *Homarus* were at 15–20°C. Probably these differences are related to the temperature by which the animals live under normal conditions. Studies on crustacean fatty-acid composition have demonstrated a characteristic annual cycle (Herodek & Farkas, 1959; Farkas & Herodek, 1959). The animals obviously are able to adapt themselves to the environmental temperature of the milieu by changing the amount of the polyunsaturated acids. The high content of polyunsaturated acids in marine fish oil and in fresh-water fish, especially in winter (Kelly *et al.*, 1958; Farkas & Herodek, 1964), will give the animals mobility and flexibility in swimming.

Although fresh-water and marine animals probably show adaptation to the environmental temperature by changing the fatty-acid composition, this phenomenon was not found for *Graphidostreptus* and *Avicularia*. These animals show a great activity at 25–30°C. Gradual lowering of the temperature to 15°C is attended by a decrease in metabolic activity. Prolonged lowering to about 15°C appeared fatal.

The hydrocarbons and other wax-like substances of *Graphidostreptus* and *Avicularia* are more highly labelled than the fatty acids. Presumably these compounds were synthesized from the fatty acids with a great number of carbon atoms because these acids are always very radioactive compared to those of shorter chain length.

The greatest number of branched-chain acids with an even and odd number of carbon atoms was shown in *Graphidostreptus*. It is possible that these acids were used to synthesize the hydrocarbons and wax-like substances. The epicuticle of the exoskeleton contains large amounts of wax, which protect the animal against desiccation (Edney, 1957).

In general the metabolism of the fatty acids in *Homarus*, *Avicularia* and *Graphidostreptus* follows the same pathways as described for *Astacus* (Zandee, 1966b). To gain more insight into the biosynthesis of the several fatty acids more detailed studies are necessary.

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