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DESATURATION OF PALMITIC ACID-1- C^{14} AND STEARIC ACID-1- C^{14} IN GAMMARUS (RIVULOGAMMARUS) ROESELII GERVAIS (CRUSTACEA, AMPHIPODA)

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Among Arthropods the synthesis of fatty acids has been studied mainly in Insects. The ability to synthetise saturated and monoenoic fatty acids and to transform stearic and palmitic acids to monoenoic acids was demonstrated in many species belonging to different Orders (ZEBE and MCSHAN, 1959; BADE, 1964; LAMBREMONT et al., 1965, 1966; SRIDHARA et al. 1966; KEITH, 1967; STEPHEN et al. 1969). The isotopic experiments however — contrary to earlier opinions — showed no signs of polyenoic fatty acid synthesis in Insects.

In Crustacea the fatty acid composition of many species, the effect of food and temperature on the fatty acid composition and the distribution of fatty acids in different lipid classes are known (HILDITCH, 1956; FARKAS and HERODEK, 1964; WOLFE et al. 1965; HERODEK and FARKAS, 1967; HERODEK, 1969; FARKAS, 1970 a, b; COLLATZ, 1969 a, b). Till now however the synthesis of fatty acids has been studied only in two Decapods, in *Astacus astacus* L. and *Homarus gammarus* L. (ZANDEE, 1966, 1967). After the injection of acetate-1-^{14}C the saturated and monoenoic fatty acids were labelled, but not the polyenoic ones.

The present work investigates to what extent can Amphipod crustaceans desaturate the palmitic acid-1-^{14}C and the stearic acid-1-^{14}C and how are these fatty acids distributed into the different lipid classes.

Material and methods

The stearic acid-1-^{14}C (REANAL, Budapest) had the specific activity of 1.379 mCi/mmole, the palmitic acid-1-^{14}C (REANAL, Budapest) 1.110 mCi/mmole.

From the labeled fatty acid 1 μCi was dissolved in 1 ml benzene and this solution was distributed on a Macherey-Nagel 640 m filter-paper disk as evenly as possible. The benzene was then carefully evaporated. The animals were collected from the Aszófő-Séd brook on the 20th October 1969. They were kept in the laboratorium in two glass aquaria. The water level was 5 cm. The water was permanently transaerated. Both aquaria contained some hundreds of animals. The plamitic acid-1-^{14}C impregnated filter-paper was placed into the one, the stearic acid-1-^{14}C impregnated filter-paper into the other aquarium. The crustaceans eat up the filter-papers in two days. For further two days they were fed on green and decaying plants originating from the sampling place. On the fourth day the animals were rinsed with pure water, blotted on filter-

paper and weighed. The fresh weights of the palmitic and stearic acid feeding groups were 26.0 g and 14.7 g respectively. Both groups were homogenized in chloroform : metanol 2 : 1 in a Biomix blendor.

The lipids were extracted according to FOLCH (1957). The distribution of the label in the lipid classes was determined from a part of the total lipid by thin layer chromatography as described earlier (HERODEK, 1968). Another portion of the total lipid was chromatographed in the same way, then from the silica gel the triglycerides were eluted three times with diethyl ether the phospholipids three times with chloroform : metanol 2 : 1 and once by methanol : ammoniumhydroxide 10 : 1. The triglycerides and phospholipids so obtained, and a further part of the total lipid were transmethylated with cc. HCl : abs. methanol 5 : 95. The methyl esters were purified by rechromatographing on silica gel G and eluted with diethyl ether. They were then subjected to thin layer chromatography on silica gel G containing 12.5 per cent silver nitrate to separate the methyl esters of the monoenoic, dienoic, trienoic and polyenoic fatty acids. The developing solvent was benzene, the bands were detected with Rhodamine B. The fatty acid composition of the bands was checked gas-chromatographically in control runnings. The methyl esters were eluted from the silver nitrate impregnated silica gel G with diethyl ether in a micro-Soxhlet apparatus for two hours, then their radioactivity was determined.

Radioactivity measurements were carried out with a USB—2 liquid scintillation detector (Office for Nuclear Engineering Equipment Pilot Plant, Warsaw). The samples were dissolved in 8 ml scintillation liquid consisting of toluene, containing 0.4 per cent 2.5-diphenyloxazole and 0.01 per cent 1,4-di[2-(5-phenyloxazolyl)]-benzene.

The gas-chromatographical analysis was carried out as described earlier (HERODEK, 1969).

Results and discussion

In the lipids of crustaceans 18.9 and 29.1 per cent of the activity of palmitic acid-1-^{14}C and stearic acid-1-^{14}C respectively, introduced by the food, was refound. The fatty acids were practically completely esterified, about two third of them incorporated into the triglycerides and one third into the phospholipids *(Fig. 1.)*.

Neither in palmitic acid-1-^{14}C nor in stearic acid-1-^{14}C feeding animals did the di-, tri-, and polyenoic fatty acids show any measurable radioactivity. The monoenoic fatty acids on the other hand had activities amounting in the palmitic acid-1-^{14}C fed group to 19 per cent, in the stearic acid-1-^{14}C fed group to 66 per cent of the total radioactivity. That only the smaller part of the palmitic acid but the greater part of the stearic acid was transformed to monoenoic acids corresponds to the fact that in the fat of Gammarus Roeselii there is more palmitic than palmitoleic but much less stearic than oleic acid *(Table I.)*.

In rats the ratios of the labeled palmitic /palmitioleic and stearic/ oleic acid incorporations are much higher in phospholipids than in triglycerides (GÖRANSSON and OLIVECRONA, 1964, 1965; GÖRANSSON, 1965 a, b). In *Gammarus Roeselii* there is no such difference between phospholipids and triglyce-

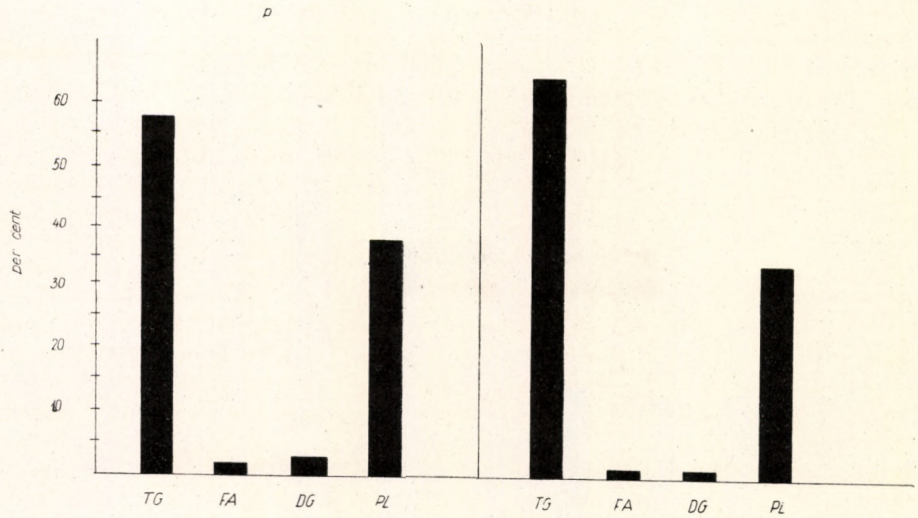

Figure 1. The distribution of radioactivity in the lipid fractions of palmitic acid-1-^{14}C and of stearic acid-1-^{14}C fed crustacenas.
Left = palmitic acid-1-^{14}C fed animals; Right = stearic acid-1-^{14}C fed animals; TG = triglycerides; FA = free fatty acids; DG = diglycerides; PL = phospholipids

TABLE 1

Fatty acid composition of the total lipid, triglycerides and phospholipids

	14 : 0	14 : 1	15 : 0	15 : 1	16 : 0	16 : 1	16 : 2	17 : 0	18 : 0
Total lipids	2.2	1.3	1.4	2.0	11.9	5.5	4.2	2.2	2.9
Triglycerides	1.4	1.2	1.0	2.0	12.2	8.5	4.6	2.1	2.8
Phospholipids	2.8	1.6	2.0	2.2	10.6	5.1	4.6	2.0	3.1

	18 : 1	18 : 2	18 : 3	18 : 4	20 : 1	20 : 4	20 : 5	22 : 5	22 : 6
Total lipids	25.8	15.3	11.0	2.5	1.4	2.6	4.0	0.4	3.4
Triglycerides	27.6	16.9	13.5	2.5	1.6	0.7	1.0	0.1	0.3
Phospholipids	19.8	13.3	8.0	1.9	1.3	5.8	8.7	1.2	6.0

The number before the colon indicates the number of carbonic atoms, that after the colon the number of double bonds.

rides. In the palmitic acid-1-^{14}C fed crustaceans 18 per cent of the radioactivity of triglycerides and 20 per cent of that of phospholipids, in the stearic acid-1-^{14}C fed crustaceans 63 per cent of the radioactivity of triglycerides and 69 per cent of that of phospholipids were in the monoenoic fatty acids *(Fig. 2.)* As compared to mammals there is more palmitoleic and much less stearic acid in the phospholipids of *Gammarus Roeselii*, therefore the saturated to monoenoic acid ratio of triglycerides and phospholipids does not differ so much in this animal.

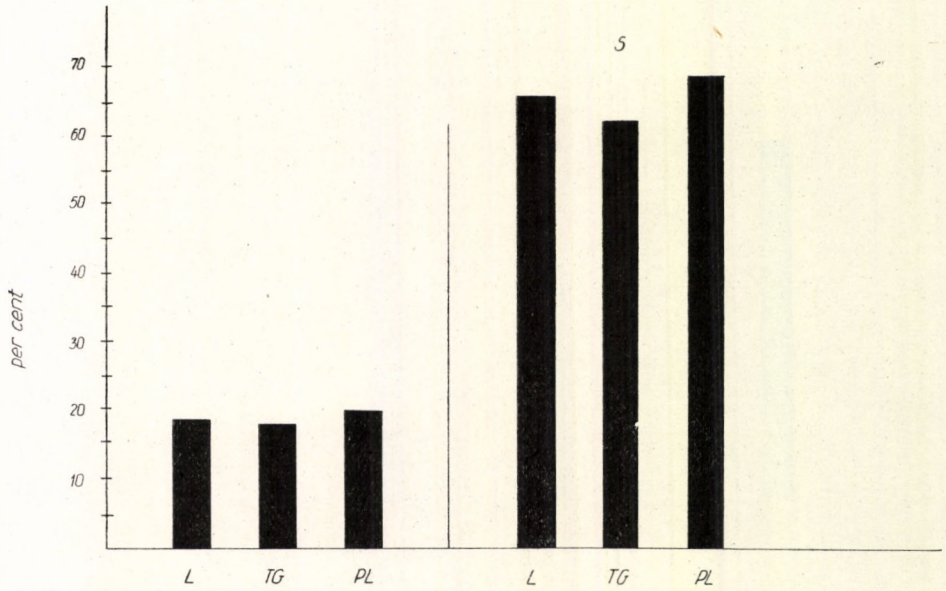

Figure 2. The radioactivity of monoenoic acids in per cent of the radioactivity of total fatty acids. L = total lipids, other abbreviations as in *Fig. 1*

The nutrition of *Gammarus Roeselii* was in detail studied by PONYI (1956, 1959). This crustacean chews with its strong mandibuls the green and decaying plants like caterpillars. Decayed plants contain a lot of cellulose. According to PONYI (1959) these animals, presumably due to their symbionts can break down cellulose. Kept only on filter-paper these crustaceans chewed it like the decaying leaves and survived longer than the control group fasting without filter-paper. This experiment gave the idea to feed crustaceans with labeled fatty acid impregnated filter-paper. Accordingly saturated and monoenoic fatty acids can be formed also from the glucose originating in cellulose. On the other hand *Gammarus Roeselii* similar to Vertebrates and Insects is not able to synthetise linoleic and linolenic acids, these compounds are taken up from green plants. The origin of highly unsaturated fatty acids of 20, 22 carbonic atoms is more problematic. These fatty acids are to be found mainly in the phospholipids, as demonstrated for several Crustacea species by FARKAS (1970 a, b). They are absent in higher plants, but may originate in epibiotic algae. It is also possible that crustaceans desaturate and elongate linoleic and linolenic acids in the way described for mammals (MEAD, 1960; KLENK and MOHRHAUER, 1960). Further feeding experiments with labeled linoleic and linolenic acids could decide whether this process exists also in crustaceans or not.

Summary

Crustaceans were fed on labeled fatty acid impregnated filter-paper for two days and on their natural food for further two days.

In crustaceans fed on palmitic acid-1-^{14}C 58 per cent of the radioactivity was incorporated into the triglycerides and 38 per cent into the phospholipids.

In the stearic acid-1-^{14}C fed group 64 per cent radioactivity was in the triglycerides and 34 per cent in the phospholipids.

In *Gammarus Roeselii* 19 per cent of the palmitic acid-1-^{14}C and 66 per cent of the stearic acid-1-^{14}C was transformed to monoenoic fatty acids.The ratio of radioactivity of saturated to monoenoic acids was similar in triglycerides and phospholipids

In the polyenoic fatty acids there was no measurable radioactivity.

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A PALMITINSAV-1-^{14}C ÉS SZTEARINSAV-1-^{14}C ÁTALAKULÁSA A GAMMARUS (RIVULOGAMMARUS) ROESELII Gervais AMPHIPODA RÁKBAN

Herodek Sándor

Összefoglalás

A rákokat két napig jelzett zsírsavval átitatott szűrőpapírral, majd még két napig a természetes táplálékukkal etettük.

A palmitinsav-1-^{14}C-vel etetett rákokban az aktivitás 58%-a a trigliceridekben, 38%-a a foszfolipidekben volt. A sztearinsav-1-^{14}C-vel etetett rákokban az aktivitás 64%-a volt trigliceridekben és 34%-a foszfolipidekben.

A palmitinsav-1-^{14}C-nek 19, a sztearinsav-1-^{14}C-nek 66%-a alakult át egyszer telítetlen zsírsavvá. A telített és monoen zsírsavak radioatkivitásának aránya a trigliceridekben és foszfolipidekben hasonló volt.

ПРЕОБРАЗОВАНИЕ I—C^{14}-ПАЛЬМИТИНОВОЙ И I—C^{14}-СТЕАРИНОВОЙ КИСЛОТЫ У РАКА *Gammarus (Rivulogammarus) Roselii Gervais* Amphipoda

Ш. Херодек

Раков кормили в течение двух суток бумагой, пропытанной меченной жирной кислотой, потом ещё в течение двух суток натуральным кормом.

У раков кормленных I-C^{14} -пальмитиновой кислотой, 58% активности было в триглицеридах, 38% в фосфолипидах. У раков, кормленных I-C^{14}-стеариновой кислотой 64% активности было в триглицеридах и 34% в фосфолипидах.

19% I-C^{14}-пальмитиновой кислоты, 66% I-C^{14}-стеариновой кислоты превращались в жирные кислоты с одной ненасыщеной связью. Соотношение радиоактивности в триглицеридах и фосфолипидах, насыщенных и ненасыщенных кислот было подобное.