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INCORPORATION OF 1-^{14}C STEARIC ACID AND 1-^{14}C LINOLENIC ACID INTO THE LIVER LIPIDS OF THE CARP (*CYPRINUS CARPIO* L.)

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In earlier in vitro and in vivo experiments the incorporation of 1-^{14}C palmitic acid was studied in tissues of carp (HERODEK, 1966; 1969a). Now tissue slices, prepared from carp liver were incubated with 1-^{14}C stearic and 1-^{14}C linolenic acids to investigate their incorporation into different lipids, and the effect of temperature on this process.

In rats a considerable amount of fatty acids is incorporated into diglycerides of long turnover time. (HERODEK, 1967; 1968; 1972). To see, whether such diglycerides are formed also in fish, a part of the liver slices incubated with labeled stearic acid was reincubated in inactive medium.

Materials and methods

Carps *(Cyprinus carpio* L.) weighing 1.5—2.0 kg, netted from Lake Balaton on the day of the experiment (18th November, 1971) were used. One gram of liver slices, prepared by rasor blade was incubated in 20 ml medium. This medium consisted of a Krebs—Ringer phosphate buffer, with a view to the lower osmotic pressure of the fish blood containing instead of 0.9 only 0.7 percent NaCl. To this solution 5 percent bovine serum albumine was added. Part of the solution was used in this form henceforth referred to as inactive medium. The other part was divided into two portions, and labeled stearic or linolenic acid was added to them. The concentration of fatty acids was 2 μmole/ml in both solutions.

The specific activity of the 1-^{14}C stearic acid (REANAL, Budapest) was 1, 379 mCi/mmole. The original specific activity of 1-^{14}C linolenic acid (UVVVR, Prague) was 450.0 mCi/mmole. This was diluted by inactive linolenic acid (Applied Science Laboratories) to obtain the needed amount of fatty acids. This way the end concentration of linolenic acid was 0.6 mCi/mmole. The fatty acids were saponified with a small excess of NaOH, and added to the preheated inactive medium. The solution was vigorously shaken and filtered.

Liver slices of the first four fish were divided into three groups. The first was incubated with labeled stearic acid for 30 min at 28° C. The second was incubated with labeled stearic acid for 30 min at 8° C. The third was incubated

with labeled stearic acid for 30 min at 28° C, then the slices were rinsed in inactive medium and reincubated in pure inactive medium at 28° C for two hours. Liver slices of the other four fish were incubated with labeled linolenic acid for 30 min at 28° C and 8° C respectively. During incubation the samples were gently shaken.

After incubation the slices were quickly rinsed in pure inactive medium then in Krebs—Ringer solution, weighed and homogenized in chloroform-methanol 2 : 1.

Lipids were extracted according to FOLCH et al. (1957). The extract was evaporated in Rotadeszt (KUTESZ, Budapest) apparatus under CO_2 atmosphere. Lipid classes were separated by thin layer chromatography, and eluted from the silica gel as described earlier (HERODEK, 1968). Lipids were dissolved in 10 ml toluene containing 4 percent PPO and 0.1 percent POPOP. The radioactivity was measured by USB-2 liquid scintillation detector (Biuro Urzadzen Technici Jadrovej).

Results and discussion

Labeled stearic and linolenic acids exhibited rather similar distribution in the lipid classes *(Table I)*. The only significant difference was found in the cholesterol esters, where the stearic acid incorporation was rather low. Temperature had no demonstrable effect on the distribution of fatty acids in lipid classes. On the contrary, the absolute amount of incorporated fatty acids depends on the type of the acid and on the temperature. Liver slices incorporated significantly ($P < 0.5$) more linolenic than stearic acid at both temperatures, and from both acids significantly ($P < 0.05$) more was incorporated at 28° C than at 8° C.

The effect of temperature on the fatty acid composition was demonstrated in microorganisms (PEARSON and RAPER, 1937; GAUGHRAN, 1947; CHRISTO-

TABLE I

The amount of 1-^{14}C stearic acid and 1-^{14}C linolenic acid in the lipid classes of liver slices after 30 min incubation at 28° C and at 8° C. (10^{-9} mole/g liver)

Labelled fatty acid	Stearic	Stearic	Stearic	Linolenic	Linolenic
Temperature °C	28	8	28	28	8
Note			reincubated in inactive medium for two hours		
Number of animals	4	4	3	4	4
Cholesterol esters	0.7±0.2	0.8±0.5	1.7±0.6	10.7±0.8	5.4±3.8
Triglycerides	33.4±7.1	18.1±9.4	27.4±7.2	45.7±4.7	27.3±3.0
Diglycerides	22.6±3.1	17.4±5.7	10.5±0.9	35.2±3.5	37.5±4.9
Phospholipids	26.2±5.4	14.9±4.9	47.7±16.1	21.4±1.6	16.1±1.5
Total esterified fatty acids	82.9±13.2	51.2±6.4	87.3±21.6	113.0±8.6	86.3±9.3
Free fatty acids	145.0±28.4	153.7±20.5	14.0±0.8	126.2±14.6	133.8±14.6
Total fatty acids	227.9±34.3	204.9±26.5	101.3±21.8	239.2±21.0	220.1±6.1

Mean ± standard error of the mean.

PHERSEN and KAUFMANN, 1955) plants (IVANOV, 1922), vertebrate and invertebrate animals (HENRIQUES and HANSEN, 1901; FAWCETT and LYMAN, 1954; THIELE, 1960). Among water organisms planktonic crustaceans, the most important natural food of fishes exhibited a rather expressed response on the effect of temperature (FARKAS and HERODEK, 1964; HERODEK, 1969 b). In these animals the amount of polyunsaturated acids, primarily that of the most unsaturated docosahexaenoic acid increased by decreasing and decreased by increasing temperature ensuring this way the steadily optimal physical state of the depot fat throughout the whole year. Of the fishes *Lebistes reticulatus*, *Salmo gairdneri*, *Gambusia affinis* and *Carassius auratus* when kept in colder aquarium contained more unsaturated fat, than under warmer conditions (KAYAMA et al., 1963; KNIPPRATH and MEAD, 1966 a; 1966 b; 1968). It was also demonstrated that in *Carassius auratus* the rate of biosynthesis of unsaturated fatty acids related to that of saturated acids increased at lower temperature (KNIPPRATH and MEAD, 1968). It is possible, that the temperature exerts its effect mainly in this way on the fatty acid composition.

However, there was an additional possibility if the temperature influenced to different degrees the rate of incorporation of saturated and unsaturated acids. Were the incorporation of stearic acid into the lipids more retarded at low temperature, than that of linolenic acid, it would lead to the accumulation of linolenic acid and its derivates, as the docosahexaenoic acid in the animals. By dividing the quantity of fatty acids esterified at 28° C by that esterified at 8° C in liver slices of the same fish, the mean value of the four fish and its standard error were 1.66 ± 0.21 in the case of stearic and 1.35 ± 0.15 in the case of linolenic acids. According to the means, lower temperature decreased more the incorporation of the saturated than that of the unsaturated acid, however owing to the high standard errors of the means more parallels were necessary to decide whether the fatty acid pattern is in fact influenced by the temperature in this way.

During the two-hour reincubation in inactive medium of tissue slices previously incubated with labeled stearic acid the radioactivity of diglycerides fell only to its half. Diglycerides are generally regarded as intermediates in triglyceride synthesis. They are formed from phosphatidic acid and completed by a third fatty acid to triglyceride. It was, however, found in rat tissues, that if they were incubated with labeled palmitic acid, then reincubated in inactive medium, during this second incubation the radioactivity of diglycerides fell only after one—two hours to its half value (HERODEK, 1967; 1968). Diglycerides of such long life are formed from endogenous fatty acids too, synthesized intracellularly from ^{14}C-acetate or ^{14}C glucose (HERODEK, 1972). Triglycerides must be synthesized through diglycerides of much shorter turnover time, it can therefore be supposed that two pools of diglycerides exist, in one the molecules are immediately transformed to triglycerides, in the other they persist for a longer period. The possible role of this second pool of diglycerides was discussed elsewhere (HERODEK, 1972). That they are to be found in carps similarly as in rats suggests the quite general nature of this phenomenon.

More than half of the radioactivity taken up by tissue slices was detected in the free fatty acids. While the amount of esterified fatty acids depended on the type of the acid and on the temperature, liver slices bound practically the same amount of free fatty acids from both stearic and linolenic acids at both temperatures.

In rat liver slices, incubated with labeled palmitic acid the amount of free fatty acids increased rapidly in the first 10 min, but changed little thereafter (VAVRECKA et al., 1966). Comparing the results of the present experiment, where incubation lasted for 30 min with results of an earlier experiment (HERODEK, 1966), where liver slices of carp were incubated for 10 min with labeled palmitic acid, it can be seen that the radioactivity of esterified lipids is about 3 times higher after 30 min incubation than after 10 min incubation the radioactivity of free fatty acids on the other hand increased but very little. About the same quantity of free fatty acids was bound by one gram rat and one gram carp liver. The exact binding site is not known. Free fatty acids were not removed by rapid rinsing, but during the two hour reincubation in inactive medium their radioactivity fell to a rather low level without a corresponding increase in the radioactivity of esterified lipids. It is therefore probable that the bulk of free fatty acids was released into the medium. This indicates rather extracellular than intracellular binding.

Summary

Liver slices of carps were incubated at 28° C and 8° C with 1-^{14}C stearic and 1-^{14}C linolenic acids for 30 min. From stearic acid at 28° C 82.9 $\pm$ 13.2, at 8° C 51.2 $\pm$ 6.4, from linolenic acid at 28° C 113.0 $\pm$ 8.6 at 8° C 86.3 $\pm$ 9.3 nannomole fatty acid was esterified by one gram liver. Temperature had no detectable effect on the distribution of fatty acids in the lipid classes.

Radioactivity of diglycerides in liver slices first incubated with labeled stearic acid decreased to its half only after a two-hour reincubation in inactive medium. This indicates some other role of diglycerides in addition to the participation in the rapid process of triglyceride synthesis.

The amount of free fatty acids bound by liver slices was independent of the type of fatty acid given and of the temperature, but decreased to a very low level during reincubation in inactive medium.

REFERENCES

CHRISTOPHERSEN, J., KAUFMANN, W. (1955): Spektraloptische und papierchromatographische Untersuchungen an Lipoiden und Fetten von Hefezellen bei verschiedenen Züchtungstemperaturen. — *Kieler milchwirtsch. Forschungsber.* **7,** 323—335.

FARKAS, T., HERODEK, S. (1964): The effect of environmental temperature on the fatty acid composition of crustacean plankton. — *J. Lipid Res.* **5,** 369—373.

FAWCETT, D. W., LYMAN, C. P. (1954): The effect of low environmental temperature on the composition of depot fat in relation to hibernation. — *J. Physiol.* **126,** 235—247.

FOLCH, J., LEES, M., SLOANE-STANLEY, G. H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. — *J. Biol. Chem.* **226,** 497—509.

GAUGHRAN, E. R. L. (1947): Saturation of bacterial lipids as a function of temperature. — *J. Bact.* **55,** 506—507.

HENRIQUES, V., HANSEN, C. (1901): Vergleichende Untersuchungen über die chemische Zusammensetzung des tierischen Fettes. — *Skand. Arch. Physiol.* **11,** 151—155.

HERODEK, S. (1966): Comparison of the triglyceride synthesis of carp and rat in adipose tissue and liver slices incubated with 1-^{14}C-palmitic acid. — *Annal. Biol. Tihany* **33,** 151—158.

HERODEK, S. (1967): The distribution of labelled palmitic acid into the diglycerides and triglycerides of rat adipose tissues. — *Lipids* **2,** 299—302.

HERODEK, S. (1968): Temporal changes in the distribution of labelled palmitic acid in the different lipids of rat's adipose tissue, liver and diaphragm. — *Acta Biochim. et Biophys. Acad. Sci. hung.* **3,** 227—237.
HERODEK, S. (1969 a): The metabolism of intracardially injected 1-^{14}C palmitic acid in the carp *(Cyprinus carpio* L.). — *Annal. Biol. Tihany* **36,** 179—184.
HERODEK, S. (1969 b): Gas chromatographic studies on the seasonal changes in the fatty acid composition of the copepod (Crustacea) plankton. — *Annal. Biol Tihany* **36,** 173—177.
HERODEK, S. (1972): Formation of diglycerides of long turnover time form labelled acetate and glucose in rat tissues. — *Lipids* **7,** 572—575.
IVANOV, S. L. (1922): The influence of climatic factors on the physiologic-chemical characters of the plants. — *Trud. Prikl. Bot.* **13,** 483—491.
KAYAMA, M., TSUCHIYA, Y., MEAD, J. F. (1963): A model experiment of aquatic food chain with special significance in fatty acid conversion. — *Bull. Jap. Soc. Sci. Fish.* **29,** 452—458.
KNIPPRATH, W. G., MEAD, J. F. (1966 a): Influence of temperature on the fatty acid pattern of muscle and organ lipids of the rainbow trout *(Salmo gairdneri).* — *Fish. Ind. Res.* **3,** 23—27.
KNIPPRATH, W. G., MEAD, J. F. (1966 b): Influence of temperature on the fatty acid pattern of mosquitofish *(Gambusia affinis)* and guppies *(Lebistes reticulatus).* — *Lipids* **1,** 113—117.
KNIPPRATH, W. G., MEAD, J. F. (1968): The effect of the environmental temperature on the fatty acid composition and on the in vivo incorporation of 1-^{14}C acetate in goldfish *(Carassius auratus* L.). — *Lipids* **3,** 121—128.
PEARSON, L. K., RAPER, H. S. (1937): The influence of temperature on the nature of the fat formed by living organisms. — *Biochem. J.* **21,** 875—879.
THIELE, O. W. (1960): Die Lipide der Weinbergschnecke *(Helix pomatia).* Über die Neutralfette und Steroide. — *Z. Physiol. Chem., Hoppe-Seiler's* **321,** 29—37.
VAVRECKA, M., POLEDNE, R., PETRÁSEK, P. (1966): Kinetics of (1-^{14}C) palmitate uptake and incorporation into lipid fractions of liver slices. — *Biochem. Biophys. Acta* **125,** 176—178.

AZ 1-^{14}C SZTEARINSAV ÉS 1-^{14}C LINOLÉNSAV BEÉPÜLÉSE A PONTY *(CYPRINUS CARPIO* L.) MÁJÁNAK LIPIDJEIBE

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Összefoglalás

A pontyok májából készített metszeteket 1-^{14}C sztearinsavval, illetve 1-^{14}C linolénsavval inkubáltuk 8 és 28 °C-on 30 percig. Egy gramm máj sztearinsavból 28 °C-on 82,9 ± 13,2; 8 °C-on 51,2 ± 6,4; linolénsavból 28 °C-on 113,0 ± 8,6; 8 °C-on 86,3 ± 9,3 mμmol-t észterezett. A hőmérséklet nem befolyásolta a zsírsavak egyes lipid csoportok közötti megoszlásának arányát.

Ha az 1-^{14}C sztearinsavval inkubált májszeleteket inaktív közegben tovább inkubáltuk, a digliceridek radioaktivitása két óra múlva csökkent csak a felére, ami azt mutatja, hogy ennek a vegyületnek más szerepe is van, mint a gyors triglicerid szintézisben való részvétel.

A májszeletek által megkötött szabad zsírsavak mennyisége nem függött sem a zsírsav fajtájától, sem a hőmérséklettől, de nagyon csökkent az inaktív közegben való reinkubálás során.