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THE METABOLISM OF THE INTRACARDIALLY INJECTED 1—^{14}C PALMITIC ACID IN THE CARP *(CYPRINUS CARPIO L.)*

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In mammals basic importance is ascribed to the blood free fatty acids for their very short turn over time in the transport of lipids and in the energy providing system of the organisms. Papers on this subject are accordingly numerous (ANNISON, 1964).

In our knowledge no experiments of this type have been carried out on fish up till now. The aim of the present study was to investigate whether after injecting labeled palmitic acid into the circulation of fish similar phenomena occur as in mammals, and that how far fatty acid transport among tissues of fish resembles to that in mammals.

Material and methods

Carps of 0.8—1.2 kg weight were used. They were transferred from the fish-pond at Irmapuszta on 29 October 1968. Before experiment fish were kept in aquarium for a day.

1-^{14}C palmitic acid (REANAL, Budapest) was used as labeled fatty acid. Its specific activity was 1 mCi/mmol. The blood plasma of one fish was separated. The labeled fatty acid was saponified by a small excess of 0.1 N NaOH. The fish blood plasma was poured to this optically clear, warm, radioactive soap solution, the mixture was vigorously shaken and filtered. The plasma obtai nedthis way contained 2 μCi/ml labeled palmitic acid bound to albumine. Of this plasma 0,5 ml i. e. 1 μCi fatty acid was intracardially injected into each fish

The injection was carried out slowly (in cca. 10 sec). The fish were then placed in a 100 liter aquarium for 5, 20 and 60 min respectively. After this interval the animals were removed and exsanguinated by cutting off their tail. The blood was immediately collected in chloroform: methanol 2 : 1. The blood obtained varied between 9 and 17 g. The liver was first rinsed in 0.49% NaCl, then weighed. An about 3 g part of the liver was cut off, weighed and homogenized in chloroform: methanol 2 : 1. In the case of the fish killed 60 minutes after the injection some flesh and some intestinal adipose tissue were also homogenized. Lipids were extracted from all tissues according to FOLCH et al. (1957). Solvents were evaporated under N_2 in Claisen apparatus, the lipids were dissolved in chloroform and stored under N_2 at —20 °C until further use.

The lipid classes were separated by thin layer chromatography as described earlier (HERODEK, 1968).

Radioactivity measurements were carried out with a Packard Tri-Carb scintillation spectrometer. The lipid samples were dissolved in 10 ml scintillation solution consisting of toluene containing 0.4 per cent 2,5-diphenyloxazole and 0.01 per cent 1,4-di-/[2-/5-phenyloxazolyl/-]benzene.

Results are expressed in per cent of the injected radioactivity, i.e. in 10^{-2} μCi. Data represent averages at 5 min of 3, at 20 min of 2 and at 60 min of 4 animals.

Results and discussion

Radioactivity of blood free fatty acids at different points of times after injecting labeled palmitic acid into fish is demonstrated in *Fig. 1.* For comparison data obtained in experiments with rats by GÖRANSSON and OLIVECRONA (1964) are also shown in this *Figure.* The disappearance of the labeled fatty acids from fish blood was so much slower that the time scale for fish

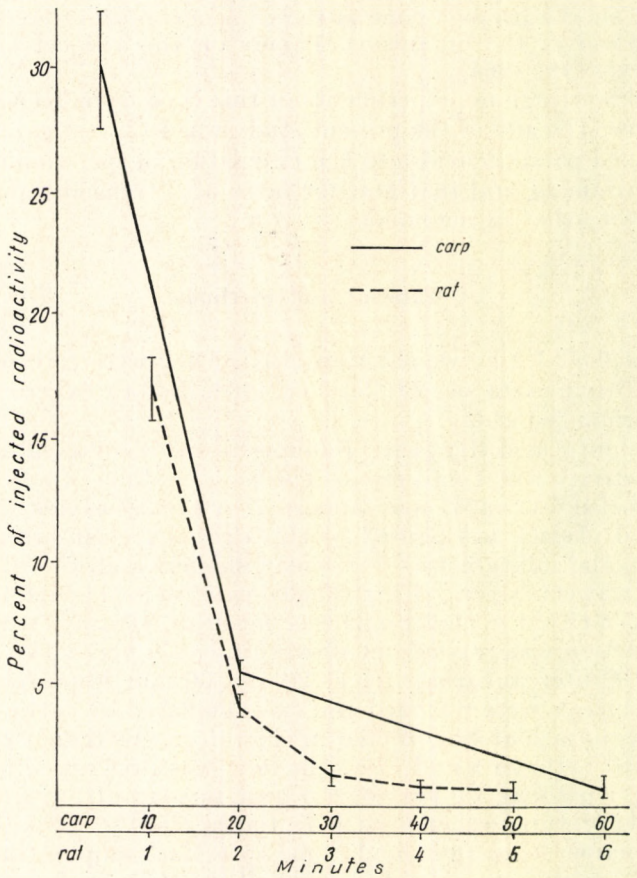

Figure 1.
Radioactivity in the blood free fatty acids after injection of 1-^{14}C-palmitic acid

had to be reduced to the tenth of that of rats to obtain curves of matching slopes. While in rats labeled free fatty acids practically disappeared from the circulation within 5 min, in carps about 30 per cent of the injected radioactivity is still present in the blood free fatty acids after the same period. In carps the radioactivity of free fatty acids decreased in 20 min to the same extent as in rats within 2 min and even after one hour a well measurable activity — about one per cent of the injected dose — was present in their blood free fatty acids. The turn over time of free fatty acids seems to be therefore in carps about ten times longer than in rats. For calculations the weight of blood was taken as 3 per cent of the weight of the whole body (KORZHUYEW and NIKOLSKAYA, 1951). The concentration of free fatty acids in the blood of carps is 0.6—1.2 μmol/ml (FARKAS, 1967) which is similar to that of mammals. From the data above it seems, that related to body weight in carps the turn over rate of blood free fatty acids is about twenty times lower than in rats. Ratio of their oxygen consumption is similar, it is in rat 2000 ml 0_2/kg/h and in carp 104 ml 0_2/kg/h *(Handbook of Biological Data,* 1956). In mammals free fatty acids of the blood originate in the adipose tissue. The adipose tissue amounts in mammals to 10—20 per cent of the body weight. The adipose tissue of fishes releases in vitro the same quantity of free fatty acids as the mammals' adipose tissue of the same weight (FARKAS, 1967). However fishes differ from mammals in having no subcutaneous adipose tissue, and that their intestinal adipose tissue amounts only to 1—2 per cent of their body weight. From this amount of adipose tissue the fatty acid inflow into the circulation is with one order of magnitude smaller than in mammals agreeing to the fact that the free fatty acid outflow of fish blood is also one order of magnitude lower due to the longer turn over time.

In mammals about one third of labeled fatty acids injected into the circulation is taken up by the liver. In liver of carps only a much lower radioactivity was detected *(Table 1).* As demonstrated above from t e blood of carp

Table 1.

Radioactivity in liver lipid fractions in per cent of the injected radioactivity

Lipid fraction	Time after injection		
	5 min.	20 min.	60 min.
Cholesterol esters	0.079 ± 0.015	0.221 ± 0.076	0.079 ± 0.013
Triglycerides	0.551 ± 0.202	3.023 ± 0.944	1.040 ± 0.204
Free fatty acids	0.654 ± 0.220	0.324 ± 0.188	0.669 ± 0.185
Diglycerides	0.246 ± 0.058	0.450 ± 0.346	0.317 ± 0.037
Phospholipids and Monoglycerides	0.733 ± 0.434	1.999 ± 0.478	1.260 ± 0.505

Mean values ± S. E. of the mean.

the free fatty acids disappear very slowly, thus even after a whole circulation time the bulk of labeled acids remains still in the blood. The liver's poor fatty acid uptake therefore can be really interpreted as a sign of a smaller or different role of the liver in the fat transport in carp than in rat. Also in carp liver

most of the labeled fatty acids are present in triglycerides and phospholipids. A considerable radioactivity is in the diglycerides observable too, as it was found already earlier in tissue slices of carp liver incubated in vitro with labeled palmitic acid (HERODEK, 1966). It seems that the formation of diglycerides of very long turn over time (HERODEK, 1967) represents a quite general phenomenon of triglyceride synthesis.

In the liver relatively much of labeled palmitic acid is present in free form. The question may arise whether these fatty acids are not those of the blood, remaining in the liver in spite of the thorough washing? In case of the group of one hour it can be unequivocally seen that these free fatty acids really belong to the liver, for at this time the total blood shows no more than 1 per cent of the injected radioactivity, and this value in the liver is 0.6.

Hardly any activity is present at first in the lipid esters of the blood after injecting labeled fatty acids into normal rats, but after 15 min their activity abruptly rises exceeding in a few minutes 1 per cent of the injected activity. In hepatectomized animals no such rise occurs, proving that these lipids are synthetized in the liver (BORGSTRÖM and OLIVECRONA, 1961). In carp blood only very low radioactivities were found in the lipid esters *(Table 2)* and in

Table 2.

Radioactivity in blood lipid fractions
in per cent of the injected radioactivity

Lipid fraction	Time after injection		
	5 min.	20 min.	60 min.
Cholesterol esters	1.357 ± 0.161	0.368 ± 0.036	0.232 ± 0.042
Triglycerides	0.353 ± 0.095	0.044 ± 0.012	0.033 ± 0.006
Free fatty acids	29.923 ± 3.640	5.231 ± 0.232	1.028 ± 0.140
Diglycerides	0.175 ± 0.034	0.054 ± 0.001	0.052 ± 0.019
Phospholipids and Monoglycerides	0.219 ± 0.056	0.126 ± 0.021	0.058 ± 0.020

Mean values ±S.E. of the mean.

contrary to the mammals, even these values decreased with time. Conceivably while in mammals great part of free fatty acids gets first into the liver, where it is incorporated into the different lipids of lipoproteins and transported in this form to the consuming tissues, in fishes the tissues take up the free fatty acids directly from the blood.

One hour after injection 1 g flesh contained 0.045 ± 0.004 per cent, and 1 g adipose tissue 0.021 ± 0.010 per cent of the radioactivity. According to this even the specific activity of flesh is higher than that of the adipose tissue, and as the total mass of the flesh is much larger than that of the adipose tissue, there must be an even higher difference in the total radioactivity uptake. The flesh of carps, living in fish ponds, may contain 10 per cent fat, demonstrating that in fishes the musculature is more important as fat depot than the adipose tissue.

Summary

Labeled palmitic acid, bound to plasma was injected into the circulation of carps, and 5, 20 and 60 minutes thereafter radioactivity of different lipid classes of the blood and liver was determined.

The blood free fatty acids contained in the 5th minute thirty per cent, in the 20th five per cent and in the 60th minute one percent of the injected radioactivity.

The liver took up much less fatty acids than usual in experiments with mammals.

The esterified fatty acids of the blood showed all the time low radioactivities.

In fish examined the turn over of blood free fatty acid was with one order of magnitude slower than in mammals. The free fatty acids seem to get directly to the consuming tissues without previous incorporation into the lipoproteins in the liver.

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A PONTYBA INTRACARDIALISAN INJEKTÁLT 1-^{14}C PALMITINSAV ANYAGCSERÉJE

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Összefoglalás

Pontyok vérébe plazmához kötött jelzett palmitinsavat juttattunk, majd meghatároztuk vérük és májuk különböző lipid frakcióinak rádióaktivitását 5, 20, ill. 60 perccel az injekció után.

A vér szabad zsírsavai a beadott dózisnak az ötödik percben 30, a huszadikban 5, a hatvanadikban 1 százalékát tartalmazták.

A máj sokkal kevesebb jelzett zsírsavat vett fel, mint amennyit az emlősök mája szokott.

A vérben az észterezett zsírsavak mindvégig alacsony aktivitást mutattak.

A vizsgált halakban a vér szabad zsírsavainak forgalma egy nagyságrenddel kisebb, mint az emlősökben. Úgy látszik, a szabad zsírsavak zömmel közvetlenül jutnak el a felhasználó szövetekhez, és nem épülnek be a májban a lipoproteidekbe.

ОБМЕН ВЕЩЕСТВ I-^{14}C ПАЛЬМИТИНОВОЙ КИСЛОТЫ ВВЕДЕННОЙ ВНУТРИСЕРДЕЧНО КАРПУ

Ш. Херодек

В крови карпа было введено связана плазмой меченная пальмитиновая кислота; затем через 5, 20 и 60 минут была определена радиоактивность различных липидных фракций крови и печени.

Свободные жирные кислоты содержат 30 процентов введённой радиоактивности через 5 минут, 5 процентов — через 20 и один процент — через 60 минут.

Печень карпа накапливает значительно меньше меченых жирных кислот, чем печень млекопитающих.

Эстеры свободных жирных кислот крови характеризовались низкой активностью в ходе всего процесса.

В изученных видах рыб обмен свободных жирных кислот ниже на один порядок, чем в млекопитающих. Вероятно, что большинство свободных жирных кислот транспортируются непосредственно к тканям, и так они не входят в состав липопротеидов печени.