Incorporation of Acetate-1-C^{14} into Liver Cholesterol of Rats Subjected to Prolonged Exercise

Following the effect of physical exercise on fat metabolism in animals, a lowering of liver cholesterol was repeatedly observed[1-4]. 24 h after cannulation of the bile duct and after collection of all bile produced, liver cholesterol is significantly lower in rats subjected to regularly repeated or a single exercise prior to cannulation[5,6]. Further experimental evidence is needed to explain these observations. Biosynthesis of cholesterol is one of the important factors in regulation of liver cholesterol.

Materials and methods. 10-week-old male white rats, whose starting weight was 225 g, were subjected to regular exercise for 168 days. The exercising group swam for 1 h daily, 6 days a week in 28 °C water. After a few days of adaptation the animals quickly adjusted to swimming. Their weight gains throughout the experiment were stable and no significant differences from the weight curves of control rats were observed. Rats of the control group were immersed regularly in the water to exclude the experimental influence of the bath. Both groups of rats were fed a natural pelleted diet of constant composition (9% fat, 30% protein, 50% carbohydrates, about 45 mg of naturally occurring cholesterol per 100 g food). For the final evaluation we had 10 rats in the control and 13 in the exercising group.

After 18 h of fasting and 24 h from the last exercise, we injected 40 μC of acetate-1-C^{14} per 100 g body weight into the peritoneal cavities of the rats both in exercising and control groups. After the injection, the animals were placed in glass metabolic cages. They were sacrificed exactly after 60 min since the application of the isotope.

Their livers were removed, and their surfaces rinsed with water and blotted. Aliquot parallel amounts of liver tissue were weighed, homogenized and extracted with chloroform-methanol[7]. This extract was used for the standard biochemical lipid analyses and for thin-layer chromatography (TLC) of lipids. Total liver cholesterol was analyzed using the LIEBERMANN-BURCHARD color reaction[8], total liver esterified fatty acids (FA) were determined[9] along with liver phospholipids[10]. The procedure of BOBERG[11], combined with the following steps, was used in TLC separation of liver lipids. Silica Gel CH Spolana 400 mesh was mixed with absolute ethanol containing 2% toluene scintillator. The extracted lipids were applied to the activated plates and the separation of phospholipids from other lipid fractions was achieved at room temperature using ethyl ether. After separation, the spots were visualized in UV-light.

Lipids, except the phospholipid fraction, were then extracted from the adsorbent by chloroform-methanol (2:1) and hydrolyzed in 0.4% ethanol solution of KOH. After neutralization (0.2N sulphuric acid) and extraction with hexane the sample was applied again to the plates coated with the adsorbent already described. The solvent mixture used was petroleum ether, hexane and ammonium hydroxide, 70:30:3 respectively. This adsorption system differentiates total cholesterol from total FA (free FA, FA bound in triglycerides and in cholesterol esters). The spots of lipid fractions detected under the UV-light were scraped into counting vials. Each sample was counted after addition of 1 ml methanol and 5 ml of liquid scintillator (toluene solution of PPO and POPOP). The quantity of radiation was counted in a Mark I Nuclear Chicago instrument at 0 °C. The quenching of the samples was corrected for by an external standard method. Radioactivity of the samples was expressed as disintegrations per min (DPM), of the respective lipid fraction isolated from 1 g of liver tissue. The significance of differences was proved using the standard Student t-test.

Results and discussion. The intensity of physical activity in the exercising group was moderate, no significant differences in body weights were observed (Table I). The tendency to higher body weight in the swimming animals was observed in several of our experiments and we attribute it to the higher proportion of lean body mass. In regularly exercising animals, liver cholesterol was lower ($P < 0.05$). The differences in other lipid fractions, viz. the total esterified FA and phospholipids, have no significance (Table I). Table II illustrates acetate-1-C^{14} incorporation into total liver cholesterol and total liver FA (except the phospholipid FA). In swimming animals the incorporation of acetate into total cholesterol is higher ($P < 0.05$), its incorporation into total FA fraction is lower ($P < 0.01$).

Table I. The influence of 1 h of swimming daily for 168 days on the body weight, liver cholesterol, liver esterified FA and liver phospholipids in rats

Group of rats	Body weight (g) (end of experiment)	Liver		
		Cholesterol (mg/100 g)	Esterified fatty acids (mEq/100 g)	Phospholipids (mg/100 g)
Resting	247.7 ± 16.9	240.2 ± 6.4	15.2 ± 1.3	151.2 ± 3.3
Swimming	289.3 ± 12.2	211.7 ± 8.6	14.8 ± 1.1	148.2 ± 5.5
Significance	$P > 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$

Means ± S.E.M.

Table II. Incorporation of acetate-1-C^{14} into total cholesterol and total FA (except FA bound in phospholipids) of the liver in swimming and non-exercising rats.

Group of rats	Acetate-1-C^{14} incorporation (DPM/1 g liver weight)	
	Total liver cholesterol	Total liver fatty acids
Resting	18,262 ± 3,096	14,327 ± 1,068
Swimming	29,825 ± 4,306	9,130 ± 1,232
Significance	$P < 0.05$	$P < 0.01$

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A lower level of cholesterol in the liver tissue of regularly swimming rats as reported here, confirms earlier findings. Lowering of liver cholesterol was noted in exercising rats fed a natural diet, as well as in animals fed a high cholesterol, high saturated fat diet[3]. The effect of exercise on lowering liver cholesterol in animals fed a high cholesterol diet which inhibits cholesterologenesis, is further evidence that exercise does not accomplish the lowering of liver cholesterol by inhibiting cholesterol biosynthesis.

The higher acetate incorporation into cholesterol, reported in this article in exercising rats, may be explained by the existence of an inverse relationship between the amount of cholesterol present in the liver and the synthesis of cholesterol from acetate. Higher acetate incorporation into liver cholesterol and its lower incorporation into liver FA were observed in animals fed diets rich in polyunsaturated FA[12,13]. We suggest that the increased incorporation of acetate into liver cholesterol in exercising rats is secondary to the lowering of liver cholesterol which may be affected by unsaturated FA released from depot fat during exercise[14].

Zusammenfassung. Inkorporation von Azetat-1-C^{14} in Lebercholesterol erweist sich bei regelmässig schwimmenden Ratten im Vergleich mit nichttrainierten Tieren als höher. Erstere hatten niedrigere Lebercholesterolwerte. Die höhere Inkorporation von Azetat ins Lebercholesterol wird mit der indirekten Beziehung zwischen Lebercholesterol und der Synthese von Cholesterol in der Leber erklärt.

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Renal Regeneration in Chloroform-Poisoned Male Mice of Strain C3H/He Treated with di-Sodium Versenate

Chloroform induces necrosis of proximal convoluted tubules in male mice of the strains C3H/He, C57BL/6JN, BN, as well as in those of generation F1 from the crossing ♀♀ C3H/He × ♂♂ C57BL/6JN[1,2]. In males of the strain C3H/He necrosis is followed by tubular calcification and death of animal within 4–8 days after poisoning[2]. In contrast to the males of all other strains mentioned above, those of the strain C3H/He fail to show any signs of tubular regeneration[2]. Assuming that tubular calcification could interfere with the regeneration we wanted to check the effect of a drug used for the elimination of calcium from the body. According to our knowledge trisodium versenate or tetra-sodium versenate are used for clinical purposes[3,4]. Since only di-sodium versenate used as decalcifier in histological laboratory was available for us we decided to use it in our experiment.

Material and methods. 15 male mice of the strain C3H/He, aged 2–3 months and weighing 18–20 g, were pretreated with a single dose of di-sodium versenate $-CH_2 \cdot N\ (CH_2COOH) \cdot CH_2COONa_2 \cdot 2H_2O$ –/2 mg dissolved in 0.2 cm^3 of physiological saline i.p. some min prior to the s.c. administration of a single dose of chloroform (0.1 cm^3 of the solution: 0.05 g of chloroform in 1 ml of ethyl laurate). The animals were killed successively after 24 h/2 mice, 48 h/2 mice (1 mouse died

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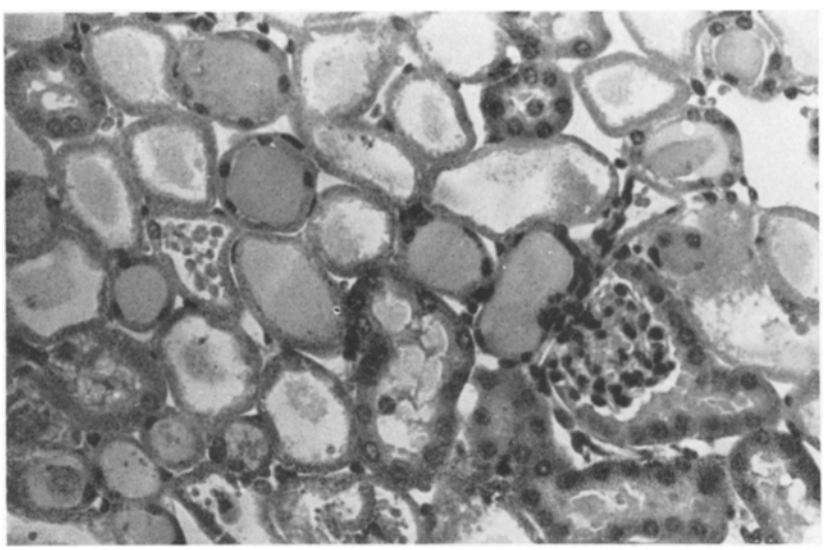

Fig. 1. Di-sodium versenate-treated male mouse of the strain C3H/He 24 h after chloroform poisoning. Tubular necrosis. H.E., × 400.