REDUCING THE DEGREE OF LIPOID INFILTRATION OF RABBIT TISSUES BY FEEDING CHOLESTEROL FREED FROM ITS OXIDATION PRODUCTS

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When kept under aerobic conditions cholesterol (ChS) readily undergoes auto-oxidation with the formation of various polar products: epoxides, ketones, hydroperoxy- and hydroxy-derivatives [12, 14]. Oxidation products of ChS have a cytotoxic action, as has been demonstrated by the use of cultures of aortic smooth-muscle cells [9] and fibroblasts [3, 7]. Administration of oxidation products of ChS to rabbits has been shown to facilitate the development of hypercholesterolemia [1, 2] and of damage to the aorta [1, 4, 8, 11, 14]. On the basis of existing data it has been suggested that damage to the aorta arising as a result of feeding rabbits with high doses of ChS [13] may be attributable not only to the atherogenic action of ChS itself, but also to the cytotoxicity of its oxidation products, which are present in commercial preparations of ChS [10, 14]. In previous investigations [1] the writers observed a marked decrease in the ChS content in atherogenic classes of lipoproteins in the blood plasma of rabbits receiving a ChS preparation free from auto-oxidation products for a long time [1, 2]. Accordingly it was interesting to study lipid deposition in the tissues of animals during chronic peroral administration of the ChS preparations having undergone different degrees of oxidation.

In this investigation histochemical and biochemical methods were used to study the degree of lipid accumulation in the liver and myocardium of rabbits fed from 12 weeks with a commercial preparation of ChS containing about 5% of its oxidation products, or with a ChS preparation free from contamination by auto-oxidation products as a result of double recrystallization from ethanol.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male Chinchilla rabbits weighing initially 2-2.5 kg, which received 0.5 ml/kg of olive oil (group 1, control), 0.2 g/kg of a commercial ChS

TABLE 1. Changes in Content of Neutral Lipids in Liver of Rabbits Receiving Olive Oil for 12 Weeks, and also of Rabbits Receiving Commercial or Recrystallized ChS Dissolved in Olive Oil (M ± m)

Class of lipids	Content of lipids, mg/g tissue		
	olive oil (5)	commercial ChS (11)	recrystallized ChS (21)
Free ChS	1,14±0,15	3,19±0,37*	2,97±0,31*
Triglycerides	1,12±0,32	1,19±0,32	1,13±0,26
ChS esters	1,38±0,09	27,90±6,62**	13,04±2,76***

Legend. *p < 0.001, **p < 0.01 compared with control; ***p < 0.05 compared with commercial ChS. Number of animals given in parentheses.

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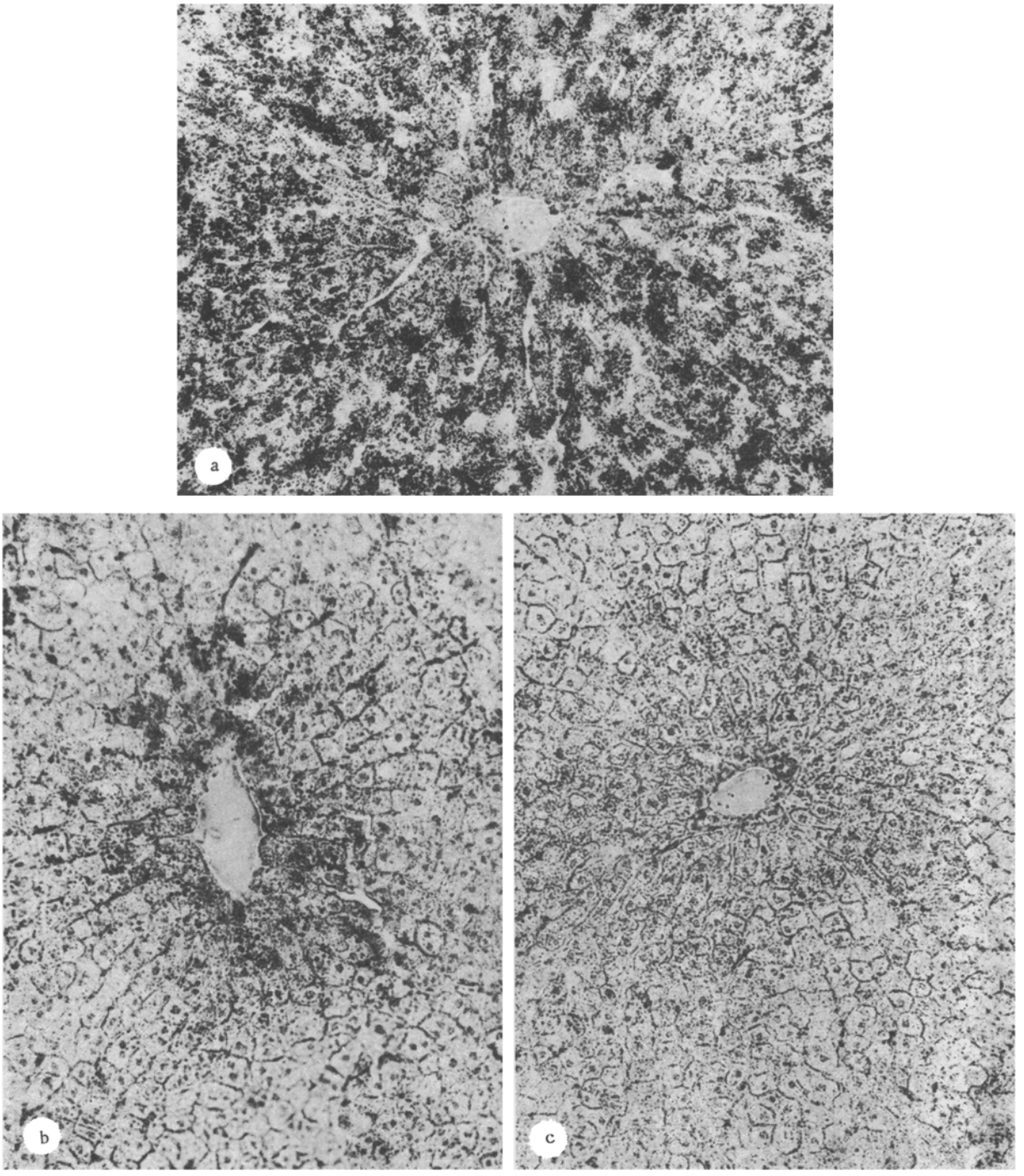

Fig. 1. Liver of rabbit receiving commercial ChS (a) and recrystallized ChS (b, c). a) Structures of lobules lost; hepatocytes no longer arranged in complexes, with deposition of tiny lipid droplets and with disintegration; large drops of fat can be seen in the central and peripheral portions of the hepatic lobules, and are located inside the Kupffer cells and also extracellularly. b, c) Structure of the lobules preserved; small quantities of lipids deposited in hepatocytes in central portion of hepatic lobules (b); lipids completely absent in hepatocytes and Kupffer cells (c). Here and in Fig. 2: Oil red and hematoxylin. 150 ×.

preparation obtained from the S. M. Kirov Leningrad Meat Combine (group 2), or an equal dose of ChS, purified by double recrystallization from ethanol (group 3), in the form of suspensions in vegetable oil. These preparations were administered daily for 12 weeks, the doses being corrected as the animals' body weight increased. At the end of the experiment the animals were killed and lipids were extracted from samples of liver by Folch's

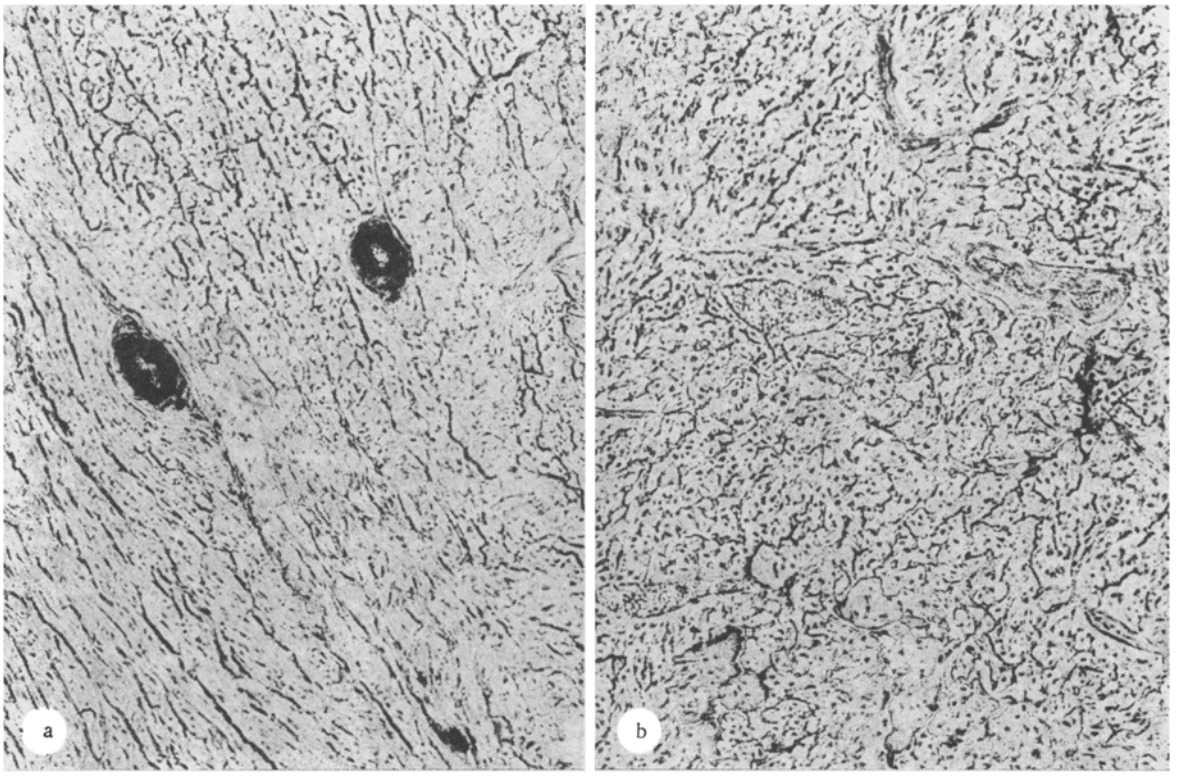

Fig. 2. Heart of rabbit receiving commercial ChS (a) and recrystallized ChS (b). a) Intramural vessels with thickened wall and concentric narrowing of lumen due to lipid accumulation in the intima and muscular coat; b) intramural arteries are unchanged.

method [5], with a mixture of chloroform and methanol (2:1 by volume), after which the lipid extracts were fractionated by thin-layer chromatography of silica-gel in a system of solvents consisting of hexane—diethyl ether—glacial acetic acid (70:30:2) on Kieselgel 60, F_{254} plates (Merck, West Germany), and the content of the lipid fractions was determined quantitatively by densitometry at 197 nm [6] on the Opton GM-3 microspectrophotometer (West Germany) with Spectra Physics 4200 integrator (USA). For the histochemical investigations pieces of liver and myocardium were fixed in 10% neutral formalin for 5 days, after which sections were cut from them to a thickness of 9 μ on the Frigomobile instrument (Reichert-Jung, Austria). Sections were stained with Oil red O and Weigert's hematoxylin. Because of the closely similar optical density of the lipid inclusions in the cellular structures in the histologic preparations of tissues from the organs studied, it was difficult to use the method of cytophotometric analysis, and accordingly the sections were studied under the light microscope, and the degree of lipid accumulation was assessed visually and expressed as follows: 0) no lipids present in the liver; +) tiny droplets of lipids deposited in hepatocytes of the inner third of the hepatic trabeculae; ++) lipids deposited in hepatocytes of the inner two-thirds of the hepatic trabeculae and Kupffer cells; +++) lipids deposited in hepatocytes and Kupffer cells over the whole extent of the trabeculae.

EXPERIMENTAL RESULTS

On macroscopic investigation of the liver of all the rabbits of group 1, which received commercial ChS for 12 weeks, it appeared flabby, and it was yellow ochre in color on section. On microscopic investigation considerable lipid accumulation was found in the cytoplasm of both hepatocytes and Kuppfer cells, progressing to disintegration of the cells and release of lipids into the extracellular space (degree of lipid deposition +++; Fig. 1a).

Macroscopic examination of the liver and the rabbits of group 2 revealed no changes compared with the group of animals receiving the solvent alone. On microscopic examination of the liver of rabbits receiving recrystallized ChS, deposition of fine droplets of liver was observed in the cytoplasm of the hepatocytes in 52% of animals in the central parts of the hepatic lobules, with only small quantities of lipids deposited in the Kupffer cells (degree of lipid deposition +; Fig. 1b), and in 48% of animals the liver showed no morphological changes (Fig. 1c).

In animals of the control group receiving olive oil no changes were found in the liver on macroscopic or microscopic investigation.

The lipid content in the liver of rabbits receiving preparations of ChS with a varied content of its oxidation products for a long time is in agreement with the results of biochemical analyses. As the results in Table 1 show, the content of esterified ChS in the liver of the animals of group 1 was more than twice as high as in the liver of the rabbits of group 2, which received recrystallized ChS. Meanwhile, the free ChS content was significantly, and virtually equally increased in the liver of the rabbits of both groups receiving the ChS preparations with different levels of oxidation products compared with the control, whereas the triglyceride content in the liver of the animals fed with the ChS preparation was unchanged compared with the control (Table 1). Lipid accumulation in the liver cells of rabbits receiving ChS preparations, which was particularly marked in animals receiving the commercial preparation of ChS, containing auto-oxidation products, according to the results of the biochemical investigation was therefore connected mainly with accumulation of ChS esters in the liver. Comparison of the results of the histochemical and biochemical investigations (Fig. 1a, b; Table 1) shows that accumulation of ChS esters was most marked in the liver of the animals fed for a long time on the commercial (oxidized) but not on the recrystallized (unoxidized) preparation of ChS.

Microscopic investigation of the myocardium revealed that in 100% of cases in the animals of group 1 massive deposition of lipids in the intima and muscular coat, both intra- and extracellularly, was observed in the walls of the intramural blood vessels of the myocardium and, in particular, of the papillary muscles and the inner layer of the myocardium (Fig. 2a). These changes were found in the myocardium of the rabbits of group 2 in only 10% of cases and they were milder in character; in 90% of cases the arterial walls contained no lipid inclusions (Fig. 2b).

The results thus indicate the possibility of considerable lipid accumulation in the liver and in the wall of the intramural blood vessels of the myocardium of rabbits fed with ChS preparations containing auto-oxidation products, whereas feeding with pure ChS either did not cause any such changes in the liver and myocardium, or did so in only solitary animals and by a much lesser degree.

The data described above are in good agreement with the results of the writers' previous investigations, which showed that hypercholesterolemia develops more rapidly in rabbits receiving ChS containing its auto-oxidation products than in those receiving pure ChS [1, 2], and also with data obtained by other workers on the cytotoxicity and angiotoxicity of ChS oxidation products [10, 11, 14]. The results of the present investigation confirm the hypothesis that ChS oxidation products are atherogenic [1, 2, 11, 14] and they point to the urgent need for future research in this direction.

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