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PATTERN OF HYPERLIPIDEMIA IN RATS WITH CHRONIC RENAL FAILURE

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The character of the hyperlipidemia was studied in rats with chronic uremia caused by removal of five-sixths of the total weight of kidney tissue. The blood cholesterol and phospholipid concentrations were almost twice the normal level 13-30 days after subtotal nephrectomy in the rats with uremia. The hyperlipidemia was more marked when the blood nonprotein nitrogen level was high. The serum triglyceride concentration was not increased. The total concentration of serum β- and pre-β-lipoproteins, determined nephelometrically, was significantly increased only if the nonprotein nitrogen exceeded 80 mg%. Disk electrophoresis of the serum lipoproteins of the rats with uremia revealed a definite increase in the α-lipoprotein concentration and a very small increase in the β-lipoprotein concentration. Postheparin plasma lipolytic activity in the rats with uremia was normal. Massive proteinuria was observed in the experimental rats, but the hypoproteinemia was not significant.

KEY WORDS: hyperlipidemia; lipoproteins; atherosclerosis; proteinuria; postheparin plasma lipolytic activity.

There is convincing evidence at the present time in support of a disturbance of lipid metabolism and the more rapid development of atherosclerosis in patients with chronic renal failure (CRF), whose life can be supported by programed hemodialysis [5, 9].

Unlike in man, atherosclerotic changes in the vessels (aorta) do not develop in rats with experimental CRF, although in the late stages sclerosis of the media caused by deposition of calcium salts may take place [15]. In view of these differences it was decided to investigate the serum lipid concentration and lipoprotein spectrum and the postheparin lipolytic activity (PHLA) of the plasma in rats with long-lasting CRF and to compare any posssible changes with the lipid disturbances in patients described previously [2, 5]. There are no data in the literature on relations between the lipoproteins and PHLA of the plasma in rats with CRF, and only scattered reports of their blood lipid concentration [4].

EXPERIMENTAL METHOD

Experiments were carried out on 44 noninbred male rats weighing initially 140-160 g, 27 of which had experimental CRF whereas 17 acted as the control. Experimental CRF was induced by removal of five-sixths of the total weight of the kidney tissue in two stages [11]. Observations were kept for 13-30 weeks after the

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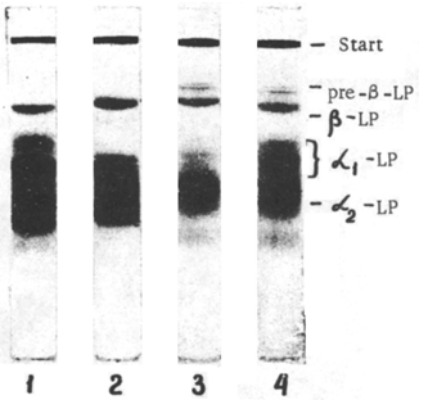

Fig. 1. Disk electrophoresis of serum lipoproteins (LP) of healthy rats (3, 4) and of rats with CRF in 7th month of experiment (1, 2).

last operation. The nonprotein nitrogen concentration in serum obtained from blood taken from the tail after starvation for 18 h was determined by Assel's method, triglycerides as in [6], cholesterol by the Liebermann–Burchard reaction after extraction with a mixture of chloroform and methanol [7], and phospholipids as inorganic phosphorus after extraction in the same way [3]. The total serum proteins were determined by the biuret method and the total β- and pre-β-lipoproteins nephelometrically [1]; the plasma PHLA was determined as described in [2]. Heparin was injected into the femoral vein in a dose of 20 units/100 g body weight. The rats were decapitated 10 min later and blood was collected in tubes with heparin (2 units to 1 ml blood). An emulsion of soy oil activated by rat serum was used as the substrate. The plasma PHLA was estimated from the accumulation of free fatty acids (FFA) in the incubation medium. The serum lipoproteins were fractionated by electrophoresis in polyacrylamide gel [12]. The protein concentration in the urine collected over a period of 18 h was determined by the sulfosalicylic acid method. Pieces of the stump of the kidney and the aorta at the level of the arch were examined morphologically. Ordinary histological survey methods of staining and the following histochemical methods were used: staining for calcium by Kossa's method, for uric acid and urates by the Schultz–Schmidt method, for elastic tissue with fuchselin, and for lipids with Sudan III and IV by Goldman's method. Neutral mucopolysaccharides were detected by Shabadash's method and acid mucopolysaccharides by staining with alcian blue and toluidine blue at pH 7.0 and 4.0.

Depending on the blood nitrogen levels the experimental rats were divided into two groups: group 1 with a blood nonprotein nitrogen of under 80 mg%, and group 2 with a level of over 80 mg%.

EXPERIMENTAL RESULTS

As Table 1 shows, a marked increase in the blood nonprotein nitrogen was observed in the rats with experimental CRF and their serum cholesterol (by 2-2.5 times) and phospholipid (by 1.5-2 times) concentrations also were considerably increased. As the blood nitrogen rose, the two last indices also showed a tendency to rise (group 2). The serum triglycerides remained at the normal level. The hyperlipidemia in experimental CRF thus differed from the uremic hyperlipidemia in man, which is characterized by an increase in the concentration of the triglycerides only, and by little change in the cholesterol and phospholipid levels [2, 5]. Since no hypertriglyceridemia was present in the rats with CRF, the increase in the total serum concentrations of β- and pre-β-lipoproteins must be attributed to β-lipoproteins participating in cholesterol transport. During disk electrophoresis of serum lipoproteins of healthy rats (Fig. 1), the following principal lipoprotein fractions were found (just as by Narayan [13] also). The fraction closest to the start (pre-β-lipoporteins) was detected inconstantly. Next followed β-lipoproteins, and then two fractions of α-lipoproteins: the principal, or α_2-lipoproteins, and an intermediate fraction of α_1-lipoproteins, consisting of two almost confluent subfractions. The band preceding the α_2-lipoproteins was formed by a complex of fatty acids with albumin transporting them.

The most characteristic change of the lipoprotein spectrum in CRF was a marked increase in the α_2-lipoproteins and a smaller increase in the α_1-lipoproteins. An increase in the intensity of the β-lipoprotein band also was observed, but this was smaller and less constant. In rats with CRF most of the cholesterol and phospholipids, just as in normal rats, is evidently transported in the α-lipoprotein fractions. This may explain the absence of any significant increase in the serum β-lipoprotein concentration (determined nephelometrically)

TABLE 1. Serum Concentrations of Lipids, β- and Pre-β-Lipoproteins, Total Protein, and Nonprotein Nitrogen, Plasma PHLA, and Proteinuria in Rats with CRF (M ± m)

Group of rats	Number of rats	Serum						Proteinuria, mg protein/ 18 h	Plasma PHLA, μeq FFA/ml/ min
		nonprotein nitrogen, mg%	triglycerides, mg%	cholesterol, mg%	phospholipids, mg%	total β- and pre-β-lipoproteins, mg%	total protein g%		
Control	17	31,7±1,6	31±2,44	65±3,8	92±5,3	48±5,3 (16)	6,8±0,1	3,8±0,4	0,52±0,01 (15)
Experimental group 1	14	60±2,9	33±4,5	134±6,0	155±9,9	64±6,8	6,4±0,1	98±19,2	
P_1			<0,05	<0,001	<0,001	>0,05	<0,02	<0,001	0,49±0,01 (15)
Experimental group 2	13	118±8,7	41±4,1	155±9,4	196±10,3	104±9,6	6,4±0,6	84±12,4	
P_1			>0,05	<0,001	<0,001	<0,001	>0,05	<0,001	>0,05
P_2				>0,05	<0,02	<0,01			

Note. 1) P_1 calculated by comparison with control group, P_2 by comparison with group 1. 2) Number of determinations differing from number of animals in group shown in parentheses.

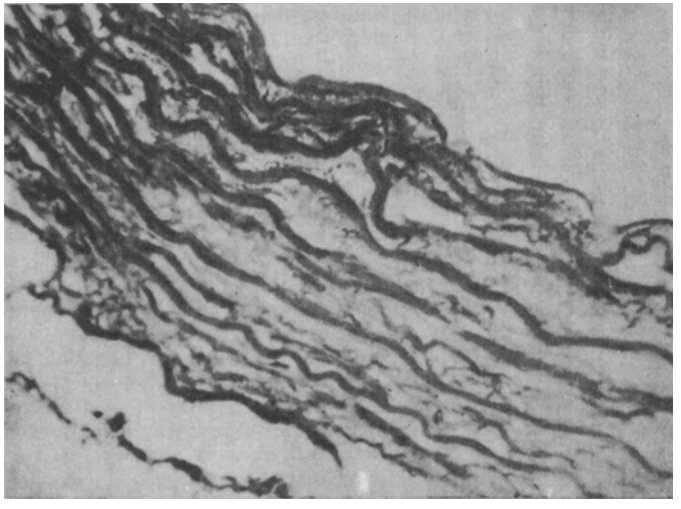

Fig. 2

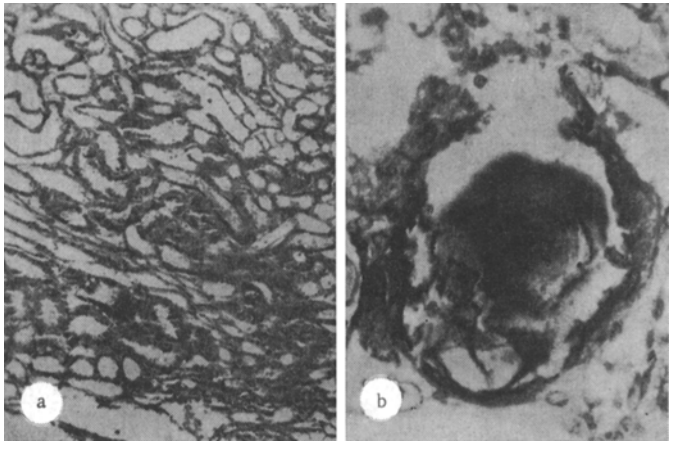

Fig. 3

Fig. 2. Aorta of rats with CRF at 7th month of experiment: disturbance of staining properties of elastic fibers. Fuchselin, 400×.

Fig. 3. Kidney stump of rat with CRF after 7 months of experiment: microliths in lumen of tubules: a) hematoxylin-eosin; 100×; b) Koss' stain, 400×.

in the rats of group 1, despite the definite hypercholesteremia. Only a further increase in the hyperlipidemia (group 2) led to a statistically significant increase in the β-lipoprotein concentration. We know that α-lipoproteins, the concentration of which rises in experimental CRF, can penetrate into the subendothelial layer of the blood vessels, but because of the small size of their molecules, they are not retained in it, i.e., these lipoproteins are not atherogenic.

In patients with uremic hypertriglyceridemia the blood concentration of coarsely dispersed pre-β-lipoproteins is increased whereas the α-lipoprotein concentration is reduced [2, 5], and this corresponds to the atherogenic type IV of hyperlipoproteinemia. It is thus possible that the absence of atherosclerotic changes in the aortas of the rats with CRF may be connected with the character of the hyperlipoproteinemia. Changes found after 7 months of the experiment (Fig. 2) affected the elastic framework of the aortic wall and were most marked in the subendothelial layer of the intima and the central zone of the media. Staining for elastin became pale or disappeared altogether. The elastic fibers swelled and thickened, and sometimes separated. In the interstitial tissue acid and neutral mucopolysaccharides were deposited. No accumulations of calcium salts or lipids were detected.

In a previous investigation [2] a definite decrease in the plasma PHLA, which includes the group of enzymes participating in triglyceride hydrolysis, was observed in patients with CRF. A decrease in PHLA was observed in the patients even in the early stages of CRF and it preceded an increase in the plasma triglyceride concentration [10]. This may partly explain the uremic hypertriglyceridemia. In the present experiments the plasma PHLA remained normal in the rats with CRF despite their high blood nitrogen. Determinations were made on 15 rats with CRF. The mean blood levels found in these animals were: nonprotein nitrogen 103 ± 10.5 mg%, triglycerides 41.1 ± 3.9 mg%, cholesterol 145 ± 7.0 mg%, and phospholipids 183 ± 8.5 mg%. The normal plasma PHLA was perhaps maintained by an increase in the concentration of α-lipoproteins, an apolipoprotein of which is an activator of lipoprotein lipase, the principal enzyme of PHLA [8]. The hyper-α-lipoproteinemia in rats with CRF, the normal plasma PHLA, and the absence of hypertriglyceridemia are thus factors not predisposing to the development of atherosclerotic changes in the blood vessels.

The mechanism of development of uremic hyperlipidemia in rats is not clear. Since one cause of the hyperlipidemia in renal disease is recognized to be a fall in the serum protein concentration due to proteinuria, these indices were determined in the rats with CRF.

As Table 1 shows, the excretion of protein with the urine of the experimental animals was sharply increased but the hypoproteinemia was not significant (group 1) or absent. It may be that in experimental CRF, just as has been postulated for the nephrotic syndrome, the synthesis of serum proteins including α-lipoproteins is increased in response to the proteinuria. As a result the normal blood protein level is maintained and hyperlipidemia develops.

Because of the massive proteinuria in the rats with CRF a morphological investigation was made of the kidney stump. The predominant picture in these experiments was vicarious dilatation of the lumen of the tubules, some of which contained calcium phosphate microliths. There was little change in the glomerular apparatus. Only in a few cases was the cavity inside Bowman's capsule enlarged and the basement membrane of the capsule irregularly thickened. No inflammatory reactions of the stroma were observed. A similar picture was found by other workers [14] who suggest that the proteinuria in experimental CRF develops as a result of "overstrain" of the basement membranes of the functioning glomeruli.

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TAUROCHOLIC ACID SYNTHESIS IN DOGS ON A HIGH CHOLESTEROL DIET

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Synthesis of ^{35}S-labeled taurocholic acid was studied in dogs with a gall bladder fistula kept on a high-cholesterol diet (2 g/kg). In the first period (1-2 months) of feeding on a high-cholesterol diet a marked increase in the synthesis of [^{35}S]taurocholic acid was observed. After 5-6 months, in the period of development of the pathological process in the liver, the synthesis and secretion of bile acids and the formation of a cholesterol residue in the bile were reduced. The serum cholesterol level in these dogs was increased but not significantly.

KEY WORDS: taurocholic acid; high-cholesterol diet.

The digestive tract plays an essential role in the synthesis, absorption, and subsequent conversion of cholesterol [9, 15]. The liver and intestine are evidently the main sources of endogenous cholesterol [5, 6, 11, 14]. If cholesterol synthesis in the liver is controlled through a system of negative feedback by the quantity of cholesterol taken in with the diet, cholesterol synthesis in the intestine, as has been shown for rats, must be independent of the amount of exogenous cholesterol ingested [10]. Exogenous cholesterol taken in with the diet has been shown to be converted in the liver into bile acids. An excessive intake of cholesterol is regulated by an increase in its conversion into bile acids and its excretion with the bile, by inhibition of cholesterol synthesis in the liver, by an increase in the excretion of cholesterol and its derivatives with the feces, and by restriction of its absorption from the digestive tract [7, 12, 15-19]. These changes are differently expressed in animals of different species, and this accounts for the differences in the degree of development of hypercholesteremia and of accumulation of cholesterol by the liver and other organs in experiments on animals receiving high-cholesterol diets. Administration of large doses of cholesterol to dogs led to a small increase in the serum cholesterol concentration, to marked accumulation of cholesterol in the liver tissue, and the increased formation of bile acids [13]. It was concluded that in animals of this species the main regulatory mechanism is the conversion of cholesterol in the liver into bile acids. However, it is not clear to what extent this mechanism operates in the presence of diseases of the liver.

The object of this investigation was to study synthesis of labeled taurocholic acid in dogs receiving a high-cholesterol diet at intervals during the development of hepatic pathology.

EXPERIMENTAL METHOD

Experiments were carried out on seven mongrel dogs with a gall gladder fistula and with the common bile duct ligated, five of which received a diet containing cholesterol (with the addition of bile of the same dog) in a dose of 2g/kg body weight daily for 6 months. On the day of the experiment the dog received 20 μCi [^{35}S]-methionine per os. The rate of excretion of radioactive label in the bile and of its incorporation into taurocholic acid was determined, and in some experiments parallel determinations were made of the cholic acid content in the bile. In a special series of experiments to determine the degree of conversion of cholesterol, entering from the intestine, into bile acids the method of isotope dilution was used. For this purpose, on the days after administration of [^{35}S]methionine bile was collected for 2-3 h, on the assumption that the quantity of radioactivity excreted in 1 ml does not change to any considerable degree, after which, when the activity of the bile was stable, the dog was given a cholesterol load and bile continued to be collected for 5 h and a

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