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Research Article

THE DISTRIBUTION OF CHOLESTEROL AND TOTAL LIPIDS IN THE NEPHROTIC RAT [1]

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The "clinical" manifestations (1) and pathological renal changes (1, 2) induced in rats by injection of rabbit anti-rat kidney serum are sufficiently similar to those observed in the human subject suffering from the nephrotic syndrome that perhaps a clear understanding of the experimental disorder might elucidate some of the puzzling aspects of its human counterpart, particularly the cholesterol and other lipid derangements so frequently observed in this disorder.

Previously, in various studies concerning cholesterol and lipid metabolism in the experimental nephrotic syndrome in rats, it was found that the characteristic hypercholesteremia observed in this disorder was of endogenous origin (3) and that it did not arise from an increased rate of cholesterol synthesis or discharge into the plasma by the liver (4) nor did it arise from any *intrinsic* failure *within* the liver to convert cholesterol into cholate (4). Subsequent studies also revealed that the hypercholesteremia was not due to either an increase in the rate of intestinal absorption (5) of cholesterol nor to a decrease in the rate of intestinal excretion of cholesterol (6).

This observed essential normality in the experimental nephrotic state of those processes concerned with the intestinal absorption and excretion of cholesterol as well as those concerned with the hepatic synthesis and conversion of cholesterol to cholate, of course, suggests that the hypercholesteremia occurring in this disorder is due to some failure in the transfer of cholesterol from the blood itself to the liver (3, 7, 8). If this last mechanism is responsible, then the nephrotic animal should exhibit an excess of cholesterol only in his blood and not in any other tissue or organ. The results of the present study suggest that indeed an excess of cholesterol accumulates only in the plasma of the nephrotic, hypercholesteremic rat.

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METHODS

A series of adult male rats was made nephrotic by injecting each with one ml. of potent rabbit anti-rat kidney serum (1). Five days later, 14 nephrotic and 5 control rats were sacrificed and each rat was completely bled from the aorta for determination of plasma total cholesterol (9) and total lipids (10). The thoracic aorta then was cannulated and the viscera thoroughly perfused with isotonic saline solution to remove as much blood as possible, the excess fluid being removed *via* the inferior vena cava. The liver of each rat then was removed and analyzed for its content of cholesterol and total lipids. In each instance, similar analysis was performed upon the combined extrahepatic, saline-washed organs of the peritoneal and thoracic cavities (intestines, kidneys, adrenals, testes, heart, lungs, spleen). The intestines were opened and thoroughly washed to remove fecal material.

Immediately after removal from the rat the tissues from each animal were weighed and refluxed with 100 ml. of a 1:3 mixture of anhydrous ether-absolute ethanol for an hour, then cut into small pieces with a long handled scissors without removing the organs from the reflux flask. Extraction was then resumed for an additional five hours, after which the extract was filtered into a volumetric flask and the organ residue and filter paper refluxed a second time for three hours with 75 ml. of the same solvent mixture. The extract was filtered into the volumetric flask and the residue extracted a third time by refluxing for six hours with 75 ml. of chloroform. The chloroform then was added to the volumetric flask and the residue extracted a fourth time with 60 ml. of chloroform. After filtration and addition of this final extract to the volumetric flask, the residue and all glassware were washed with hot 1:3 alcohol-ether. The combined extracts and washings were made up to volume. Aliquots of appropriate size were removed, placed in 25 ml. volumetric flasks and evaporated to dryness at 60°C. under nitrogen. The dry lipids were then estimated by the chromic acid oxidation procedure of Bragdon (10).

Total cholesterol was determined upon aliquots of the combined extracts and washings by evaporating at 75°C. until the odor of chloroform was no longer detectable. At this stage, about 1 ml. of fluid remained. To this fluid an equal volume of acetone was added, together

with an additional 2 ml. of 1:1 ethanol-acetone mixture. The solution was hydrolyzed by adding 10 drops of 50 per cent aqueous KOH and heating to 40°C. for 40 minutes in an air bath. After cooling, the hydrolysate was made neutral to phenolphthalein by titrating with 10 per cent acetic acid, and an additional drop of acetic acid was added in excess. The solution was filtered and the glassware and residue on the filter paper washed twice with 3 ml. of 1:1 alcohol-acetone. Total cholesterol was determined on the combined filtrate and washings by precipitation with digitonin and assay as previously described (9).

RESULTS

Marked hyperlipemia and hypercholesteremia were observed in the nephrotic rats, as shown in Table I. Analyses of their livers indicated that the contents of cholesterol as well as of total lipids were essentially the same as those observed in the control rats. Thus, the livers of the nephrotic rats contained an average of 0.323 gm. (Range: 0.266 to 0.455) of total lipids and 19.0 mg. (Range: 12.8 to 23.0) of cholesterol, whereas the livers of the control rats had an average content of total lipids of 0.315 gm. (Range: 0.282 to 0.347) and of total cholesterol, 17.4 mg. (Range: 14.0 to 19.5). Statistical analysis shows that these differences are not significant. The combined extrahepatic viscera of the nephrotic rats contained an average of 0.675 gm. (Range: 0.365 to 1.1) of total lipids and 32.9 mg. (Range: 28.0 to 36.5 mg.) of cholesterol. These values in the control rats were, respectively, 1.002 gm. of total lipids (Range: 0.775 to 1.170) and 36.2 mg. (Range: 32 to 43.0) of cholesterol. Statistical analysis again reveals these differences to be slight in regard to the total lipid content and insignificant for the total cholesterol content.

DISCUSSION

The inability in the present experiments to detect any significant deviation from normal in the cholesterol content of the nephrotic rat's liver suggests that the hypercholesteremia observed in the nephrotic state is not initiated by any change in rate of hepatic conversion or destruction of cholesterol. Thus, if there were a decreased hepatic destruction of cholesterol with a consequent "piling up" in the plasma, an increased cholesterol content might be expected in the liver. Furthermore, the failure to detect any significant change in the cholesterol content of the various viscera of the nephrotic rat suggests that none of these organs plays a provocative role in the pathogenesis of nephrotic hypercholesteremia.

In previous studies it was found that the endogenous hypercholesteremia of the nephrotic rat (3) cannot be ascribed either to an increased he-

TABLE I

Distribution of cholesterol and total lipids in nephrotic rats

	Type of rat	
	Nephrotic	Control
Number of rats	14	5
Average weight (*gm.*)	202	195
Total lipids		
Plasma (*gm./100 ml.*)	1.559 (0.730–3.960)†	0.315 (0.220–0.420)
Liver: Wet wt. (*gm.*)	8.80 (6.99–9.71)	7.85 (6.82–8.89)
Conc. (*gm./100 gm.*)	3.240 (3.020–3.700)±0.09‡	4.017 (3.925–4.125)±0.47
Content (*gm.*)	0.323 (0.266–0.455)±0.016	0.315 (0.282–0.347)±0.15
Viscera:* Wet wt. (*gm.*)	16.0 (13.7–17.2)	16.9 (14.7–18.6)
Conc. (*gm./100 gm.*)	4.258 (2.520–7.200)±0.55	6.017 (4.500–7.250)±0.66
Content (*gm.*)	0.675 (0.365–1.100)±0.08	1.002 (0.775–1.170)±0.096
Total cholesterol		
Plasma (*mg./100 ml.*)	451 (289–489)	51 (44–63)
Liver: Conc. (*mg./100 gm.*)	228 (190–262)±10.9	222 (207–238)±7.3
Content (*mg.*)	19.0 (12.8–23.0)±0.9	17.4 (14.0–19.5)±1.4
Viscera:* Conc. (*mg./100 gm.*)	207 (175–235)±6.2	216 (186–233)±12.3
Content (*mg.*)	32.9 (28.0–36.5)±0.9	36.2 (32.0–43.0)±2.8

* Myocardium, lungs, spleen, kidneys, adrenals, testes, intestinal wall.
† Range of values.
‡ Standard error of the mean.

patic synthesis of cholesterol or decreased conversion to cholate (4), or to an increased intestinal absorption (5) or decreased intestinal excretion of cholesterol (6). The present data show that the elevated cholesterol level of the nephrotic rat is *confined* to the plasma. These results suggest that, similar to other hypercholesteremic disorders, biliary obstruction (11, 12), dietary hypercholesteremia in the rabbit (13) and detergent-induced hypercholesteremia (14), nephrotic hypercholesteremia appears to be due to some failure in the normal process by which cholesterol leaves the blood and enters the hepatic parenchymal cell. This is in accord with an earlier finding (3) that cholesterol feeding in nephrotic rats leads to a *further* retention of the absorbed cholesterol in the plasma. Whether this apparent "trapping" and accumulation of cholesterol within and only within the plasma is due to some intrinsic physico-chemical change occurring between it and various plasma constituents (7, 8) or whether it is due to some failure in the cholesterol transfer mechanism existing between hepatic parenchymal cell and the plasma (*e.g.*, the Kupffer cell system [15, 16]) cannot be determined by the present experiments. It seems reasonable to believe, however, that the former possibility is the more likely, in that the hepatic R-E system is concerned primarily with exogenously derived cholesterol (15, 16), whereas nephrotic hypercholesteremia is of endogenous origin, (3). Whether the excess cholate also found in the plasma in the nephrotic state (17) is partly responsible for the apparent failure of the normal egress of cholesterol from the blood to the liver is not known at the present time.

Finally, the tendency of the total lipid dynamics to resemble those of cholesterol alone in the present and in the previous studies (3, 6) suggests that a similar retention in the plasma of the nephrotic animal may account for the rise of other lipid fractions.

The genesis of hyperlipemia and hypercholesteremia in the nephrotic human subject in many respects appears similar to that of the nephrotic rat. Thus, Stanley and Thannhouser (18) found no deficiency in the absorption of neutral fat in a nephrotic patient. In a patient with clinical nephrosis, London, Sabella, and Yamasaki (19), using tracer methods, found that the half-life time of her serum cholesterol was prolonged. Moreover, Hiller, Linder, Lundsgaard, and Van Slyke (20) and Ling and Liu (21) showed that in nephrosis the body seemed able to metabolize fats normally, but unable to remove them from the blood. Stanley and Thannhouser (18) administered labelled fat to a nephrotic patient and found a much slower than normal rate of utilization. Soshea and Farnsworth (22) interpreted their data as showing the presence of an increased lipid transport pool in nephrotic patients. These findings again suggest that the fundamental disturbance in the fat metabolism of nephrotic patients is a failure in the transfer of fats, including cholesterol, from the blood to the liver or tissue depots.

SUMMARY

The distribution of cholesterol and total lipids in the liver and extrahepatic viscera was studied in nephrotic and control rats. It was found that the nephrotic rat has a normal content of cholesterol and total lipids in its liver and other viscera, despite the marked isolated elevation of cholesterol and total lipids in its plasma.

The significance of these data was discussed in relation to other studies of experimental nephrotic rats. It was suggested that nephrotic hypercholesteremia and hyperlipemia result from a failure in the normal transfer mechanism of cholesterol and other lipids from the plasma to the liver, with consequent "trapping" and accumulation only within the plasma.

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