Effects of Altered Thyroid Function on Plasma Lipids of Experimentally Nephrotic Rats.* (24071)

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Occurrence of hypercholesteremia, increased tolerance to thyroid administration, and decreased metabolic rate in the nephrotic syndrome early implicated the thyroid gland in its pathogenesis(1). However, although the serum protein bound iodine is diminished in nephrotic patients(2), no actual abnormality of thyroid function appears to exist(3-5). The diminished protein bound iodine content of nephrotic serum appears to be due largely to its external renal loss(3-5), but also is believed by some to be partly due to deficiency of serum protein for its intravascular transport(3). It therefore appeared of interest to study the effects on nephrotic hyperlipemia of excess thyroid hormone administered in a *preformed* protein binding, and to determine the effects of preexisting thyroid dysfunction on plasma lipid changes occurring during subsequent induction of the experimental nephrotic state.

Methods. I. Effect of preexisting hyper- and hypothyroidism upon plasma lipids of rats injected with antikidney serum. A series of 32 male adult rats (Long-Evans strain) was made hyperthyroid by addition of 0.25% powdered thyroid substance to the stock ration for 18 days, and another series of 32 rats was made hypothyroid by addition of 0.3% powdered propylthiouracil to the diet for 6 weeks(6). The remaining 18 rats served as controls. Each rat was injected with 1 ml of pooled rabbit anti-rat kidney serum (AKS) (7). Groups of the hyperthyroid, hypothyroid, and control rats were sacrificed 24, 48 and 120 hours after injection of the AKS, at which time they were bled from the aorta for determination of plasma total lipids(8), total cholesterol(9), phospholipids(10), and albumin(11). Sections were obtained from the kidneys of the 16 rats sacrificed at the 48-hour interval after AKS injection and were stained with the Rinehart and Abul-Haj's modified Hale stain.

Results. Twenty-four hours after injection of AKS, the control rats exhibited average plasma total lipids of 464 mg/100 ml, total cholesterol of 133 mg/100 ml, and phospholipids of 220 mg/100 ml. These values were considerably higher and lower, respectively, in the hyper- and hypothyroid rats, despite their comparable injection of AKS (Table I). Similar differences were observed among the various groups of rats sacrificed 48 and 120 hours after AKS injection. Average plasma albumin concentrations were comparably lowered by injection of AKS in hyperthyroid and control rats, but were relatively less diminished in hypothyroid groups of rats.

Histological examination of the kidneys of the rats sacrificed 48 hours after AKS injection revealed characteristic glomerular changes and precipitated protein in the tubules(7), the changes observed in the hyper- and hypothyroid rats being of essentially comparable nature and degree to those of the control AKS-injected rats.

II. Effect of thyroid administration on plasma cholesterol of rats with preexisting nephrotic hyperlipemia. A. The effect of thyroid administration in nephrotic rats was studied by inducing the hyperthyroid state in 2 different ways. In the first experiment this was accomplished by parenteral administration of L-triiodothyronine. A series of 20 rats was injected with 1.0 ml pooled antikidney serum (AKS) and 7 days later the nephrotic rats were bled for plasma total cholesterol. Twelve of the rats then were injected subcutaneously with 100 μg of L-triiodothyronine daily for 4 days and on the fifth day were again bled for plasma total cholesterol. The remaining 8 rats served as controls. B. In the second experiment 10 additional rats were made nephrotic by injection of 1.2 ml of AKS and were bled for

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TABLE I. Effect of Pre-Existing Hyper- and Hypothyroidism on Plasma Lipids of Rats Injected with Antikidney Serum.

Type of rat	No. of rats	Avg wt (g)	Avg plasma concentrations: Total lipids (mg/100 ml)	Total cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Albumin (g/100 ml)
Rats bled 24 hr after AKS inj.						
Hyperthyroid	17	180	741 ± 53.4* (400–1270) †	186 ± 9.0 (137–226)	397 ± 51.8 (253–546)	.7 ± .09 (.4–1.0)
Hypothyroid	15	230	267 ± 18.1 (161–402)	94 ± 4.7 (67–112)	151 ± 13.6 (138–237)	1.6 ± .19 (.8–2.4)
Control	13	236	464 ± 48.2 (230–790)	133 ± 8.1 (98–196)	220 ± 37.2 (191–264)	.7 ± .10 (.5–.9)
Rats bled 48 hr after AKS inj.						
Hyperthyroid	5	190	1261 ± 112.2 (1109–1534)	237 ± 19.7 (203–284)	459 ± 64.0 (378–615)	.8 ± .25 (.7–.9)
Hypothyroid	6	224	504 ± 86.2 (208–712)	135 ± 21.4 (71–231)	181 ± 25.2 (118–250)	1.5 ± .23 (1.0–2.0)
Control	5	212	1413 ± 254.1 (466–2035)	170 ± 21.5 (100–240)	255 ± 29.3 (167–350)	.9 ± .19 (.7–1.1)
Rats bled 5 days after AKS inj.						
Hyperthyroid	10	191	2112 ± 297.0 (586–3291)	330 ± 53.3 (183–464)	646 ± 95.2 (446–850)	.9 ± .05 (.8–1.0)
Hypothyroid	11	225	644 ± 65.3 (200–987)	211 ± 21.5 (110–333)	295 ± 17.6 (224–356)	1.6 ± .04 (1.5–1.8)

* Stand. error of mean. † Range of values.

plasma total cholesterol one week later. During the ensuing 8 days, 0.4% powdered thyroid substance was added to the ration of 5 of the rats, the remaining rats serving as controls. At the end of the experimental interval the rats were again bled for plasma total cholesterol.

Results. A. Injection of L-triiodothyronine did not appear significantly to affect plasma cholesterols of the rats with preexisting nephrotic hypercholesteremia. Thus although the average plasma total cholesterol of the treated rats fell during the experimental interval from an initial average of 323 mg/100 ml (Range: 222-502) to a final average of 158 mg/100 ml (Range: 75-219), a comparable decrease was observed in the control rats whose plasma cholesterol similarly fell during the experimental interval from an initial average of 371 mg/100 ml (Range: 170-493) to 189 mg/100 ml (Range: 91-278).

B. Addition of a relatively large amount of thyroid substance to the ration of the second series of rats with preexisting nephrotic hypercholesteremia possibly exerted a greater effect than did L-triiodothyronine. Thus the plasma total cholesterol of the treated rats fell from an initial average of 628 mg/100 ml (Range: 451-816) to 168 mg/100 ml (Range: 167-176), representing an average decrease of 460 mg/100 ml (Range: 281-640; S.E. Mean ± 84.6). At the same time average plasma cholesterol of the control rats fell from 414 mg/100 ml (Range: 256-577) to 274 mg/100 ml (Range: 31-439; S.E. Mean ± 67.6). However, although the difference in the 2 groups was statistically significant, cholesterol changes in both groups of rats varied so considerably that observed results are questionable.

III. Effect of repeated administration of serum obtained from hyperthyroid rats on plasma lipids of nephrotic rats. The inconclusive effect of induced hyperthyroidism in the latter experiments may have been partly ascribable to absence in the nephrotic rat's plasma of adequate protein for intravascular hormone transport(3). To determine the possible effect of administering to nephrotic rats an excess of thyroid hormone in a *preformed* protein binding was of interest.

Methods. Hyperthyroidism was induced in a group of rats by adding 0.4% powdered thyroid substance to their diet for 21 days. The

TABLE II. Effect of Repeated Injection of Hyperthyroid Rats' Serum on Plasma Lipids of Nephrotic Rats.

Type of inj. rat serum	No. of rats	Avg wt (g)	Avg plasma concentrations: Initial — Total lipids (mg/100 ml)	Initial — Total cholesterol (mg/100 ml)	Final — Total lipids (mg/100 ml)	Final — Total cholesterol (mg/100 ml)	Final — Albumin (g/100 ml)
Rats with moderate nephrotic hyperlipemia							
Hyperthyroid	5	176	465 (175–790)*	142 (117–196)	531 (405–1000)	96 (68–170)	
Control	5	184	445 (230–710)	122 (111–137)	517 (250–810)	104 (60–161)	
Rats with severe nephrotic hyperlipemia							
Hyperthyroid	5	182		314 (234–435)	882 (584–1260)	281 (219–369)	.8 (.5–1.0)
Control	5	190		316 (176–420)	1001 (892–1150)	262 (205–366)	.7 (.4–.9)

* Range of values.

rats then were bled and their pooled serum found to contain a protein bound iodine content of 16.8 μg/100 ml in contrast to a concentration of 4.0 μg/100 ml in a second pool of rat serum obtained from untreated normal rats. A series of 10 rats then was injected with 1.0 ml of AKS. One week later these nephrotic rats were bled for plasma total cholesterol and total lipids. Five of the nephrotic rats then were injected daily intravenously with 3 ml of the pooled donor hyperthyroid rat serum, the remaining 5 control rats being similarly injected, but with the pooled serum obtained from the normal rats. Six days later the rats were bled for determination of plasma total lipids and total cholesterol. A similar experiment was performed in a second series of 10 rats in which a more severe hyperlipemic state was induced by injection of 0.75 ml AKS on each of 2 consecutive days. One week later, 5 of the rats were injected daily with 3.0 ml of the pooled hyperthyroid rat serum and the remaining 5 control rats with the pooled normal rat serum, as in the first series of rats. Plasma total cholesterol was determined prior to the first serum injection and plasma cholesterol, total lipids and albumin after 6 daily injections of the donor serum.

Results. The results are presented in Table II. The slight changes observed in both series of nephrotic rats injected with the pooled serum obtained from the donor hyperthyroid rats were closely similar to those occurring in control rats injected with normal rat serum.

Discussion. The acute administration of excess thyroid hormone, despite its route, did not appear to alter the elevated plasma lipids of nephrotic rats under the present experimental circumstances. A similar lack of apparent response to prolonged thyroid therapy has been noted in nephrotic human subjects (12). This failure of thyroid administration to lower the elevated lipid content of nephrotic plasma might be ascribed in part to renal loss of administered hormone(3-5) and to the fact that, despite the occurrence in such plasma of subnormal content of protein bound iodine (2), normal thyroid function is present in the nephrotic syndrome(3-5). Conversely, administration of excess thyroid hormone is known to be capable of lowering the plasma lipid content of normal human subjects(13), as well as that of normal rats(6). Therefore, the present failure of thyroid exhibition to lower the elevated lipid content of the nephrotic rat's plasma also suggests that there is a metabolic block in the plasma egress of the various lipid fractions, which cannot be overcome by administration of excess thyroid hormone.

Our previous studies of the mechanism of nephrotic hyperlipemia have shown that such a metabolic block in the plasma egress of lipid does indeed occur in the nephrotic state(14, 15). These studies have indicated that the

critical deficiency of circulating albumin in nephrotic plasma is primarily responsible for failure of normal lipolysis and plasma clearance of triglyceride, which results in intravascular accumulation of excess triglyceride(16-20), and associated secondary plasma retention and accumulation of excess phospholipid, cholesterol, and cholic acid characteristic of such plasma(19-20).

On the other hand, administration of excess thyroid hormone to *normal* rats was shown in previous studies from this laboratory to so markedly increase their rate of plasma elimination of cholesterol that, despite the concomitant markedly increased rate of cholesterol synthesis in such hyperthyroid rats, their resultant plasma content of cholesterol is lowered(6). This would also appear to be true of the plasma phospholipid content in the hyperthyroid state(21,22). Therefore, if a metabolic block in the intravascular egress of cholesterol and phospholipid were to be introduced during an existing state of hyperthyroidism, it might be anticipated that their markedly increased rates of synthesis consequent to the hyperthyroid state(6,21) would lead to progressive hypercholesteremia and hyperphospholipemia. This sequence was indeed found to occur in the present studies when such a metabolic block to lipid egress from the plasma was introduced by injection of antikidney serum(19). Thus it was observed that the prior presence of an established hyperthyroid state markedly augmented the rate of rise of plasma cholesterol and phospholipid otherwise occurring during early development of the nephrotic syndrome, a response which could not be ascribed to any apparent effect of the hyperthyroid state on renal changes or degree of hypoalbuminemia effected by injection of the antikidney serum in these rats.

A considerably slower rise of plasma lipids of lesser magnitude occurred in hypothyroid rats injected with antikidney serum, despite occurrence in such rats of glomerular changes of comparable nature and degree to those observed in the control and hyperthyroid rats similarly injected with antikidney serum. Although this altered response might be partly ascribable to the slowed rate of lipid synthesis in the hypothyroid state(6), it is probably mainly a consequence of the fact that less severe depletion of the plasma albumin content occurred in these rats. The results agree with earlier findings that, although elevated plasma lipid levels are inversely related to lowered plasma albumin content of nephrotic plasma (18,19), plasma lipid-albumin concentrations in such plasma are not a linear function, significant hyperlipemia occurring only in the presence of *severe* depletion of the circulating albumin content.

Summary. 1) The effect of a preexisting state of hyperthyroidism and of hypothyroidism on plasma lipid changes occurring during development of experimental nephrotic syndrome was studied in rats. Conversely, the effect of excess thyroid administration on plasma lipids of rats with previously established nephrotic hyperlipemia was studied. 2) Under present experimental circumstances, acute administration of excess thyroid hormone, orally, parenterally or intravenously in a preformed protein binding, did not significantly affect the elevated plasma lipids of nephrotic rats. On the other hand, the rise of plasma lipids occurring during development of nephrotic state in rats was markedly augmented in presence of preexisting hyperthyroidism, and conversely, was considerably slowed by prior induction of an hypothyroid state. The mechanism of these responses was briefly discussed.

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Bicarbonate Reabsorption along Renal Tubules.* (24072)

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Montgomery and Pierce first demonstrated that in the frog kidney urine is acidified in the distal tubule(1). More recently it has been suggested that the mechanism of acidification is one involving an exchange of hydrogen for sodium. Such a system is also invoked in reabsorption of bicarbonate from tubular urine. Pitts(2) and Berliner(3) suggest that hydrogen exchanges for sodium along the entire nephron, *i.e.*, in proximal as well as distal tubules. Nicholson(4) demonstrated by histochemistry that the brush border of proximal tubules may be quite acidic, suggesting that hydrogen ions are secreted into urine by proximal cells as well as distal. Using the stop flow technic devised by Malvin, Wilde and Sullivan(5,6,7), we have located along the nephron the transport systems for bicarbonate.

Principle of stop flow analysis. Administration of an osmotic diuretic mannitol to a dog establishes such a high rate of urine flow that concentrations are changed to a new steady value. If during this period the ureter is clamped shut, intratubular pressure rises until filtration at the glomeruli ceases. During this interval of stopped flow urinary concentrations will be altered as static columns of tubular urine remain trapped in the various nephron segments. If glucosuria is developed before occlusion, such that glucose reabsorptive transfer maximum is exceeded, the occlusion will allow further time for lowering glucose concentrations along the proximal tubule. If para-aminohippurate (PAH) is included in the infusion, the occlusion will allow further time for increasing the proximal PAH concentrations. In the distal areas occlusion will allow the Na concentration to be lowered. On release of occlusion the diuretic flush washes the concentration pattern along each nephron. Serial urine samples segment this pattern into an ordered array, best pictured on a graph if concentration of each sample is plotted against accumulated volume of urine which has appeared before the particular sample since reinstatement of flow. Plots against time show a distortion due to variable flow rates. The first samples to enter the collector come from distal areas and show decreasing concentrations of Na. As proximal fluid begins to enter, the Na concentrations rise, while glucose

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