Role of the Liver in the Glycoprotein Mobilizing Property of Parathyroid Extract.* (30381)

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In addition to its well known effects on Ca^{++} metabolism, parathyroid extract (PTE) increases serum glycoproteins and alters serum protein and glycoprotein fractions of rats (fall in serum albumin and rise in α_1, α_2 and β globulins)(1). At the same time glycoprotein material is deposited in the kidneys. The significance of these changes is not clear. The elevation in serum glycoproteins has been attributed to depolymerization of bone matrix following bone demineralization(2). However, as Shetlar has pointed out, one would expect to find an increase in serum uronic acids if the high levels of serum glycoprotein came from bone matrix since these are abundant in tissue(3). Instead, the increase involves mainly protein bound hexoses, hexosamine, and sialic acid; the latter is not an important component of tissue glycoprotein. In addition, recent studies on the biosynthesis of serum glycoproteins show quite clearly that the liver is the only site of synthesis of these compounds(4,5). Likewise, elevations in serum glycoproteins brought about by non-specific injury have been shown to result from active hepatic synthesis rather than from liberation of pre-formed components of tissue ground substances(6,7).

The experiments reported herein were designed to find out whether the increase in serum protein bound carbohydrate following administration of PTE to rats is of hepatic origin or simply represents a release of preformed material from such a source as bone matrix. Parathyroidectomized rats were used for the study since we had found in earlier experiments that parathyroidectomy enhances the glycoprotein mobilizing property of PTE (8) in addition to enhancing its Ca^{++} mobilizing action(9). Our data will show that it is the former view which best fits the facts.

Materials and methods. Fifty-eight male Wistar rats weighing from 150 to 190 g were divided into 5 groups and were treated as follows: Group I: Cautery parathyroidectomy (PTX) and laparotomy under ether anesthesia. During the laparotomy the liver was manipulated in order to achieve, insofar as possible, a *sham* hepatectomy. This was followed immediately by the first of 3 injections of 100 U of PTE (Lilly) given at 8-hour intervals. Group II: Cautery PTX and a 70% hepatectomy according to the method of Higgins and Anderson(10). The amount of liver left *in situ* was sufficient to insure excellent survival of the animals since no deaths from the procedure occurred during the experiment in any of the 24 hepatectomized animals. The same schedule of PTE injections as in Group I was followed. Group III: Sham PTX and 70% hepatectomy. The rats received sham injections according to the same schedule as for PTE. Group IV: Sham PTX and laparotomy followed by *sham injections* as above. Group V: no treatment.

During the experiments the rats were fasted and had access only to tap water. All rats were sacrificed 30 hours after the beginning of the experiment by exsanguination from the inferior vena cava under ether anesthesia. The serum was separated immediately and frozen. The kidneys were removed and placed in 10% buffered formalin.

The following analyses were carried out on the serum samples. (1) Total protein using a biuret reaction(11). (2) Total protein bound hexose (glycoproteins) measured by the tryptophane method of Shetlar *et al*(12). (3) Total protein bound sialic acid measured by the method of Warren(13) after preliminary hydrolysis (1 hour at 80° in 0.1 N HCl) followed by phosphotungstic acid precipitation. (4) Serum protein and glycoprotein fractions were measured by paper strip electrophoresis with bromophenol blue and periodic acid Schiff (PAS) staining for protein and glycoprotein fractions respectively. Strips were scanned with the Beckman "analytrol" using

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TABLE I. Effect of PTE and Hepatectomy on Serum Proteins and Glycoproteins and Serum Ca^{++}.

	Group 1	Group 2	Group 3	Group 4	Group 5
No. rats	14	14	10	10	10
Serum protein (g/100 ml)	5.69 ± .37	5.13 ± .33	4.74 ± .51	5.38 ± .26	5.76 ± .35
Serum glycoprotein (mg/100 ml)	221.6 ± 22.9	155.7 ± 21.9 p <.001	128.7 ± 22.3 p*<.001	169.2 ± 14.5	160.3 ± 11.6 pt<.001
Serum protein bound sialic acid (mg/100 ml)	173.7 ± 19.6	113.4 ± 15.6 p <.001	89.5 ± 16.3 p*<.001	124.0 ± 11.9	130.7 ± 11.4 pt<.001
Glycoprotein to protein ratio (g hexose/100 g protein)	3.89 ± .28	3.02 ± .33 p <.001	2.71 ± .32 p*<.01	3.14 ± .23	2.79 ± .21 pt<.001
Serum Ca^{++} (mEq/l)	6.86	7.49 p <.05	4.66 —	5.0	—

Group 1: PTE; Group 2: PTE + hepatectomy; Group 3: hepatectomy; Group 4: laparotomy; Group 5: no treatment. Rats of Groups 1 and 2 also had parathyroidectomy.
All values = mean ± S.D.
p = significance of Δ between means of Groups 1 and 2; p* = significance of Δ between means of Groups 3 and 4; pt = significance of Δ between means of Groups 1 and 5.

the appropriate filters. (5) Serum Ca^{++} was measured by the method of Zak and expressed as mEq/l(14).

Histological sections of the kidney specimens (rats of Groups 1, 2 and 5 only) were prepared with the following stains: (1) Hematoxylin and eosin (H & E); (2) Von Kossa's stain for calcification; (3) PAS stains for neutral glycoprotein. Reading of the slides was carried out in a blind manner. Renal calcification as well as deposition of PAS positive material were graded on a 0 to ++++ basis.

Student's t test was used for statistical analysis of the data.

Results. Serum proteins. As shown by others(1), total serum protein levels were not altered by PTE (Table I). After hepatectomy alone the serum proteins were slightly lower than after a sham hepatectomy. The principal action of PTE in rats of Group 1 was a redistribution of serum proteins (fall in serum albumin and increases in the a_1, a_2 and β globulin fractions) (Table II). These PTE induced changes in serum protein fractions were absent in hepatectomized rats of Group 2. In these rats the values for these fractions were similar to those found for Groups 4 and 5.

Serum glycoproteins. In rats of Group 1, PTE produced the characteristic rise in serum glycoproteins measured by protein bound hexoses (Table I). This rise was completely blocked by hepatectomy. After hepatectomy alone (Group 3) there was a fall in serum glycoproteins by comparison with sham-treated control animals (Group 4) Serum glycoprotein levels in rats having had hepatectomy and PTE were slightly higher than after hepatectomy alone.

Changes in serum glycoprotein fractions in rats of Group 1 were identical to those described by others(1) after PTE administration: fall (not significant) in albumin glyco-

TABLE II. Effect of PTE and Hepatectomy on Serum Protein Fractions.

	Group 1	Group 2	Group 3	Group 4	Group 5
No. rats	14	13	10	10	9
Albumin	2.16 ±.50	2.54 ±.25 p<.01	2.82 ±.24 p*>.2	2.88 ±.34	3.45 ±.43 pt<.001
a_1	1.34 ±.18	.93 ±.15 p<.001	.49 ±.07 p*<.001	.69 ±.09	.81 ±.15 pt<.001
a_2	.86 ±.28	.42 ±.13 p<.001	.38 ±.21 p*>.2	.44 ±.12	.54 ±.16 pt<.01
β	1.08 ±.25	.75 ±.12 p<.001	.62 ±.13 p*<.01	.86 ±.19	.59 ±.12 pt<.001
γ	.25 ±.13	.54 ±.17	.42 ±.18	.51 ±.28	.43 ±.29

Group 1: PTE; Group 2: PTE + hepatectomy; Group 3: hepatectomy; Group 4: laparotomy; Group 5: no treatment.
All values = mean ± S.D. in g/100 ml.
p = significance of Δ between means of Groups 1 and 2; p* = significance of Δ between means of Groups 3 and 4; pt = significance of Δ between means of Groups 1 and 5.

TABLE III. Effect of PTE and Hepatectomy on Serum Glycoprotein Fractions.

	Group 1	Group 2	Group 3	Group 4	Group 5
No. rats	14	13	10	10	9
Albumin	34.7 ± 13.0	23.8 ± 5.4 p<.01	24.0 ± 6.7 p*<.001	44.3 ±10.3	39.6 ± 9.0 p†<.2
α_1	86.0 ± 15.0	67.3 ±13.0 p<.01	51.6 ±12.0 p*>.2	55.5 ± 6.0	68.9 ±12.4 p†<.01
α_2	42.6 ± 10.7	22.1 ± 4.2 p<.001	18.7 ± 4.0 p*<.01	25.3 ± 5.3	25.0 ± 4.2 p†<.001
β	52.4 ± 7.7	33.5 ± 7.6 p<.001	27.1 ± 7.0 p*<.05	36.1 ± 9.3	21.5 ± 6.4 p†<.001
γ	5.7 ± 3.0	9.4 ± 2.6	7.3 ± 2.4	7.4 ± 4.8	6.1 ± 3.8

Group 1: PTE; Group 2: PTE + hepatectomy; Group 3: hepatectomy; Group 4: laparotomy; Group 5: no treatment.

All values = mean ± S.D. in mg/100 ml.

p = significance of Δ between means of Groups 1 and 2; p* = significance of Δ between means of Groups 3 and 4; p† = significance of Δ between means of Groups 1 and 5.

protein and rise in α_1, α_2 and β glycoprotein fractions (Table III). These changes did not occur in hepatectomized rats. However, the α_1, α_2 and β glycoprotein fractions were greater in hepatectomized rats given PTE than after hepatectomy alone.

Serum protein-bound sialic acid levels. We found that PTE had similar effects on protein bound sialic acid: a marked elevation by comparison with rats of Group 5. This elevation failed to occur in hepatectomized rats of Group 2. Protein bound sialic acid values were lower in these rats than in control animals (Groups 4 & 5) but were higher than in rats subjected to hepatectomy alone (Table I).

Carbohydrate to protein ratio. Because of the rise in protein bound carbohydrate without a change in serum proteins a corollary of the action of PTE is a rise in the carbohydrate to protein ratio. This was found in the rats of Group 1 by comparison with control rats of Groups 4 and 5. Again, this change failed to occur in the hepatectomizedd rats of Group 2 (Table I).

Serum calcium. PTE had the usual hypercalcemic action on the rats of Group 1. This was not abolished by hepatectomy and Ca^{++} levels in rats of Group 2 were actually slightly higher than in those of Group 1 (Table I). Therefore, hepatectomy caused a dissociation between the Ca^{++} mobilizing and glycoprotein mobilizing properties of PTE. In the rats of Group 3 subjected to hepatectomy alone, Ca^{++} values were slightly below normal presumably due to the lower serum protein levels in this group of rats.

Renal calcification and deposition of mucoprotein. With the doses of PTE that were used in this study, gross calcification does not become apparent until 48 to 72 hours of treatment and at 24 hours microscopic calcification is only minimal. However, parathyroidectomy as previously described, considerably enhances the nephrocalcinosis(9). Rats of Group 1 all had grossly visible renal calcification and severe microscopic calcification as evaluated by Von Kossa staining. Renal calcification was equally severe in hepatectomized rats of Group 2 (Table IV).

Parathyroidectomy also enhances the renal deposition of glycoprotein produced by PTE (8). In rats of Group 1 the formation of glycoprotein casts in the renal tubules was severe. Surprisingly, rats of Group 2 despite low serum glycoprotein values also had severe renal deposition of glycoprotein although to a slightly lesser degree than rats of Group 1.

Discussion. Both the Ca^{++} and glycoprotein mobilizing properties of PTE have been attributed to one biochemical mechanism. It has been theorized that PTE administration enhances depolymerization of bone matrix with decrease of its mineral binding capacity and increased serum Ca^{++}(2). The carbohydrate residues would enter the circulation causing elevated serum glycoprotein levels

TABLE IV. Effect of PTE and Hepatectomy on Calcium and Glycoprotein Deposition in the Kidney.

	Group 1 (14)	Group 2 (13)	Group 5 (9)
Ca^{++}	+++	+++	0
PAS	+++	++	0

Grades (from 0 to ++++) are averages of grades given to each section by "blind" examination.

and ultimately deposition of this material in the kidneys as glycoprotein tubular casts. The latter appear to precede renal calcification. In support of this thesis, Bradford *et al*(15) have found that in rats having received sodium sulfate-S^{35} PTE produced an elevation in non-dialyzable radioactivity in the serum and an increase in radioactivity in the kidney. In recent studies(8) we found that parathyroidectomy enhanced both the Ca^{++} and the glycoprotein-mobilizing properties of PTE(8), which suggests that these actions share a common biochemical mechanism. This view was supported by our finding that Actinomycin-D, which had been discovered by Rasmussen to block the Ca^{++} mobilizing property of PTE, also blocked its glycoprotein mobilizing action(16).

However, as pointed out earlier, the most serious objection against the thesis that the elevated protein bound carbohydrate in serum comes from degraded bone matrix is the absence of a rise in acid mucopolysaccharides in the serum following PTE administration. Moreover, recent work has clearly shown that in other situations such as injury which is also accompanied by increased serum glycoproteins, the latter appear to come from the liver(6,7). The sharp drop in serum glycoproteins produced by partial hepatectomy in the rat(17), the complete lack in incorporation of labelled glucose or glucosamine into serum glycoprotein in the absence of the liver suggest that biosynthesis of these macromolecules occurs in the liver(5).

The data reported herein suggest that such is the case also for the rise in serum glycoprotein following PTE administration since in hepatectomized rats given PTE (Group 2) there was a striking reduction in the levels of serum glycoprotein measured by protein bound hexose and sialic acid by comparison with rats of Group 1. If the PTE induced elevation in serum glycoprotein came only from bone matrix, one would not expect it to be completely suppressed by hepatectomy such as was the case here. In the rats of Group 3 having had only hepatectomy, the serum glycoprotein levels were, as expected, lower than in rats of Group 4 subjected to sham hepatectomy. This has been found by others(17) and is presumably due to removal of the principal or only source of biosynthesis of serum glycoproteins. Serum glycoprotein levels were slightly higher in hepatectomized rats given PTE. This was probably due to the action of PTE on the remaining liver.

The changes in serum protein and glycoprotein fractions (fall in albumin and rise in α_1, α_2 and β globulin fractions) were also completely suppressed by hepatectomy. This suggests that the abnormal distribution of protein between albumin and globulin fractions so characteristic of PTE treatment in rats is also of hepatic origin.

The surprising finding in this study was that the renal deposition of mucoprotein produced by PTE was not suppressed by hepatectomy despite a lowering of serum glycoprotein levels. Previously, it had been postulated that the large deposits of glycoprotein in the kidneys of rats receiving PTE are mobilized from the bone matrix and transported *via* the blood to the kidney. It was therefore of great interest to find that despite actual lowering of serum glycoprotein levels in rats of Group 2 per comparison with control Groups 4 and 5, there was increased deposition of PAS positive material in the renal tubules of these animals. Findings with this technic do not warrant any conclusions beyond the obvious one that the glycoprotein material in the tubules of animals receiving PTE may be formed in part by the kidney. The suppression by hepatectomy of the PTE-induced elevation of serum glycoprotein may be interpreted in two ways. It is possible that PTE may directly affect the hepatic cell and promote biosynthesis of serum glycoproteins. On the other hand, it is possible that under the action of PTE a substance is released from the bone matrix which in turn acts upon the liver to promote increased hepatic synthesis of serum glycoprotein.

Summary. Studies were carried out to determine the action of hepatectomy on the glycoprotein action of PTE as well as on PTE-induced renal calcification and deposition of glycoprotein. Our data showed that in hepatectomized rats, PTE no longer caused an elevation in serum glycoproteins. However, hepatectomy did not alter the forma-

tion of glycoprotein tubular casts produced by PTE administration. The data suggest that the liver is the major source of the elevated serum glycoproteins found in rats receiving PTE. In addition, it is possible that PTE may have a direct stimulating action on biosynthesis of glycoprotein by the kidney.

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Biochemical and Histochemical Changes in Aorta of Chicks Fed Vegetable Oils and Cholesterol. (30382)

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We reported earlier(1) that feeding vegetable oils and cholesterol to chicks for 8 weeks increased total plasma cholesterol, β-lipoprotein cholesterol, β-lipoprotein percentage, triglycerides, and free fatty acids in plasma. The rise in plasma cholesterol was maximum in chicks fed sesame oil with cholesterol or hydrogenated groundnut oil with cholesterol, minimum in chicks fed mustard oil with cholesterol with intermediate value when fed coconut oil with cholesterol. Hypercholesteremia did not depend on saturation or unsaturation of the oils. Clinical and experimental evidences indicate a direct relationship of atherosclerosis to the altered lipid metabolism characterized by hypercholesteremia and hyper β-lipoproteinemia. The origin of lipid in atheromatous plaques might be due to filtration from circulating plasma (2) or arterial synthesis of cholesterol(3-5) and phospholipids(6). Significant quantities of mucopolysaccharides containing hexosamine present in the arteries(7) are increased in rabbits fed cholesterol(8). Earliest change observed in the development of atherosclerosis was the marked accumulation of acid mucopolysaccharides(9).

We wished to investigate the interrelation, if any, between dietary fat, plasma lipids and changes in the various parts of the aorta. The distribution of elastic tissue, acid mucopolysaccharides and lipids was, therefore, determined histochemically and cholesterol, phospholipids, hexosamine and hydroxyproline were determined chemically in the different segments of the aorta in chicks fed vegetable oils and cholesterol.

Materials and methods. Two-week-old male white Leghorn chicks were fed a basal diet(10) for 3 weeks, then divided into groups of 6 birds (Table I). Oils when fed were mixed with the diet at 10% level. Cholesterol when administered was mixed with the diet at 1% level. Average daily food consumption by each chick was 30 g. After the diets were fed for 8 weeks, birds were killed