ally exist in the living vessel wall remains to be determined.

Summary. Extracts of human aortic intima were made in 0.9% NaCl and in 12% NaCl. Ultracentrifugally isolated human serum beta and alpha-2 lipoproteins were placed in similar salt concentrations. These were then examined in the immunoelectrophoresis system using antiserum against serum beta lipoprotein (which is equally effective against alpha-2 lipoprotein). Extract of aorta gave 2 lipoprotein arcs with this antiserum, one in the beta and one in the pre-albumin region, when prepared with 0.9% NaCl; there was only one arc (in the alpha-2 region) in extracts with 12% NaCl. This is interpreted as suggesting that serum alpha-2 lipoprotein is bound in the aortic intima in a pair of complexes both of which can be disrupted with strong salt solution.

1. Tracy, R. E., Merchant, E. B., Kao, V. C., Circulation Res., 1961, v9, 472.

2. Kayden, J. H., Seegal, B. C., Hsu, K. C., J. Clin. Invest., 1959, v38, 1016.

3. Hanig, M., Shainoff, J. R., Lowy, A. D., Science, 1956, v124, 176.

4. Amenta, J. S., Waters, L. L., Yale J. Biol. and Med., 1960, v33, 112.

5. Bihari-Varga, M., Gergely, J., Giro, S., J. Athers. Res., 1964, v4, 106.

6. Anderson, A. J., Biochem. J., 1963, v88, 460.

7. Oncley, J. L., Walton, K. W., Cornwell, D. J., J. Am. Chem. Soc., 1957, v79, 4666.

8. Ott, H., Lohss, F., Gergely, J., Klin. Wochenschr., 1958, v36, 383.

9. Giro, S., Gergely, J., Jakob, L., Szekely, J., Virag, S., J. Athers. Res., 1961, v1, 88.

10. Bottcher, C. F. J., Woodford, F. P., Klynstra, F. B., ibid., 1962, v3, 24.

11. Watts, H. F., Evolution of the Atherosclerotic Plaque, ed., Richard J. Jones, Univ. of Chicago Press, 1963, p117.

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Enhancement by Parathyroidectomy of Glycoprotein Mobilizing Property of Parathyroid Extract.* (30054)

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In the absence of the parathyroid glands the effects of bovine parathyroid extract (PTE) on Ca^{++} metabolism of rats are enhanced. Ca^{++} elevation is more pronounced, metastatic calcification is more severe and the animals die rapidly from the general toxic effects of PTE(1). Parenthetically this enhancement of the Ca^{++} mobilizing action of PTE by parathyroidectomy (PTX) is a useful phenomenon for bioassay of parathyroid hormone preparations(2). The effect of PTX on other properties of PTE has not been studied. In addition to mobilizing Ca^{++}, PTE increases serum glycoproteins and alters serum protein and glycoprotein fractions of rats (fall in serum albumin and rise in a_1, a_2 and β globulins)(3). Concurrently mucopolysaccharide material is deposited in the kidneys and the amount of mucoprotein in the gastric mucosa(4) increases. The significance of these changes is not known. They have been attributed to depolymerization of bone matrix following bone demineralization (5). The purpose of the studies described here was to find out if the effects of PTE on mucopolysaccharide metabolism are also enhanced in absence of the parathyroids.

Materials and methods. One hundred thirty male Wistar rats, weighing from 150 g to 180 g, were divided into 4 groups and were treated as follows: *Group I*: Cautery PTX under ether anesthesia. This was followed immediately by the first of 1, 2, or 3 injections of 100 U of PTE (Lilly) given at 8-hour intervals. Rats were sacrificed at 8, 16 or 30 hours after the beginning of the experiment, having thus received 100, 200, or 300 U of PTE respectively. *Group II*: Sham operation under ether anesthesia. The

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TABLE I. Effect of Parathyroidectomy on PTE Induced Mobilization of Ca^{++} and Glycoprotein.

Group	8 hr			16 hr			30 hr		
	I	II	III	I	II	III	I	II	C
No. of rats	11	12	11	12	11	11	27	26	9
Serum Ca^{++} (mEq/l)	7.2 ± .5 p <.001	5.8 ± .2	4.2 ± .5	8.1 ± .5 p <.001	6.4 ± .3	4.1 ± .3	7.4 ± .8 p <.001	8.4 ± .6	5.2 ± .1
Serum proteins (g/100 ml)	5.5 ± .3	5.2 ± .3	5.3 ± .2	5.7 ± .4	5.4 ± .2	5.7 ± .4	5.9 ± .5	5.8 ± .4	6.0 ± .4
Serum glycoproteins* (mg hexose/100 ml)	152.2 ±8.3 p <.01	142.1 ±5.2	149.2 ±5.9	174.1 ±11.3 p <.05	159.4 ±16.5	154.9 ±8.6	206.7 ±19.9 p <.001	179.6 ±18.9	152.2 ±11.5
CHO to protein ratio† (g hexose/100 g protein)	2.8 ± .1 p >.2	2.7 ± .2	2.8 ± .1	3.0 ± .2 p >.2	2.9 ± .3	2.7 ± .1	3.5 ± .3 p <.001	3. ± .2	2.5 ± .1
Serum sialic acid‡ (mg/100 ml)	108.0 ±6.9 p <.01	100.2 ±5.7	103.1 ±9.0	131.8 ±10.3 p >.2	123.8 ±18.9	116.2 ± .7	165.7 ±7.9 p <.01	135.4 ±12.8	109.2 ± 6.9
Tissue sialic acid‡ ($\mu M \times 10^{-3}$/mg)	1.7 ± .5 p <.2	1.4 ± .4	1.7 ± .6	2.3 ± .3 p <.1	1.9 ± .4	2.1 ± .8	3.3 ± 1.9 p <.01	2.0 ± .8	1.4 ± .4

All values = mean ± S.D. p = significance of difference between means of Groups I and II. Group I, PTX + PTE; Group II, sham operation + PTE; Group III, PTX; Group C, sham operation alone.
* Measured as protein bound hexoses. † Glycoprotein to protein ratio. ‡ Calculated as N-acetylneuraminic acid.

same schedule of PTE injections as in Group I was followed. *Group III*: Cautery PTX under ether anesthesia. Rats received injections of similar amounts of saline solution. These animals were not observed beyond 16 hours. Nine rats had a sham operation and were sacrificed at 30 hours. These served as normal controls and will be designated below as belonging to *Group C*. During the experiments, rats were allowed free access to tap water and Purina laboratory chow. The numbers of rats sacrificed at 8, 16 and 30 hours in Groups I, II, III and C, respectively, are listed in Table I. The experiment was terminated for each rat by exsanguination from the inferior vena cava under ether anesthesia. Serum was separated immediately and frozen. Kidneys were removed and placed in 10% buffered formalin. The stomach was removed from each rat and all the glandular mucosa was scraped off the underlying muscularis mucosa. Each mucosal sample was immediately frozen and lyophilized.

The following analyses were carried out on serum samples: 1) Total protein using the biuret reaction(6). 2) Total protein-bound hexose measured by the tryptophane method of Shetlar *et al*(7). 3) Serum sialic acid was measured by the method of Warren(8) after preliminary hydrolysis (1 hour at 80°) in 0.1 N HCl followed by phosphotungstic acid precipitation and was expressed as mg/100 ml. 4) Serum protein and glycoprotein fractions were measured by paper strip electrophoresis with bromophenol blue and periodic acid-Schiff (PAS) staining. Strips were scanned with the Beckman analytrol using the appropriate filters. 5) Serum Ca^{++} was measured by the method of Zak *et al*(9) and expressed as mEq/l.

Histological sections of the kidney specimens were prepared with the following stains: Hematoxylin and Eosin (H&E); Von Kossa's stain for calcification; periodic acid-Schiff (PAS) stain for neutral mucopolysaccharides.

Concentration of sialic acid in lyophilized gastric mucosal scrapings was measured by the method of Warren after 1-hour hydrolysis in 0.1 N H_2SO_4 at 80°C and expressed as $\mu M \times 10^{-3}$/mg of freeze dried tissue. The concentration of sialic acid in gastric mucosa

TABLE II. Effect of Parathyroidectomy on PTE Induced Changes in Serum Protein Fractions at 30 Hours.

Group	No. rats	Albumin	α_1	α_2	β	γ
I	25	2.55 ±.54 p <.001	1.06 ±.22 p <.001	.95 ±.29 p <.001	.88 ±.23 p <.05	.49 ±.23 —
II	25	3.07 ±.42	.78 ±.19	.71 ±.16	.77 ±.15	.49 ±.24
C	9	3.39 ±.28 p^1<.05	.68 ±.14 p^1<.2	.52 ±.11 p^1<.01	.70 ±.12 p^1>.2	.71 ±.39 —

Group I, PTX + PTE; Group II, Sham operation + PTE; Group C, Sham operation alone.
All values = mean ± S.D. in g/100 ml. p = significance of difference between means of Groups I and II. p^1 = significance of difference between means of Groups II and C.

TABLE III. Effect of Parathyroidectomy on PTE Induced Changes in Serum Glycoprotein Fractions at 30 Hours.

Group	No. rats	Albumin	α_1	α_2	β	γ
I	27	33.6 ± 8.1 p <.20	71.9 ± 13.1 p <.001	42.9 ± 9.5 p <.01	48.5 ± 8.1 p <.001	9.8 ± 4.0
II	26	36.2 ±12.4	59.1 ± 12.1	35.6 ± 6.7	39.1 ± 7.9	9.6 ± 3.0
C	9	38.4 ± 7.3 p^1>.20	48.1 ± 6.3 p^1<.02	25.6 ± 4.2 p^1<.001	27.0 ± 5.1 p^1<.001	13.0 ± 4.4 —

Group I, PTX + PTE; Group II, Sham operation + PTE; Group C, Sham operation alone.
All values = mean ± S.D. in mg/100 ml. p = significance of difference between means of Groups I and II. p^1 = significance of difference between means of Groups II and C.

was considered a measurement of mucosal content of glycoprotein.

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

Results. 1.) *Serum Ca^{++}.* As described previously(1), PTE at first caused significantly higher serum Ca^{++} levels in PTX animals than in sham-operated rats receiving identical doses of PTE. However, after this more rapid initial rise, PTX rats at 30 hours had significantly *lower* serum Ca^{++} levels than rats of Group II. Rats of Group III had the usual post PTX hypocalcemia at 8 and at 16 hours (Table I).

2.) *Serum proteins.* Serum protein levels were not altered by PTE either in normal or in PTX rats. However, the PTE-induced fall in serum albumin and increase in α_1, α_2, and β globulin fractions described previously (3) were more pronounced in PTX animals than in sham-operated rats receiving identical doses of PTE. Although the differences were apparent at 8 and 16 hours, they were statistically significant only at 30 hours (Table II).

3.) *Serum glycoproteins.* The characteristic rise in serum glycoproteins caused by PTE, as evidenced by protein-bound carbohydrates, was significantly greater in PTX rats of Group I than in sham-operated rats (Table I). Values were significantly different as early as 8 hours after the beginning of the experiment. Changes in serum glycoprotein fractions (fall in albumin glycoprotein and rise in α_1, α_2 and β glycoprotein fractions) were greater in PTX rats than in animals of Group II. These differences were statistically significant only in rats sacrificed at 30 hours (Table III).

4.) Under the influence of PTE, serum sialic acid levels increased by a significantly (except at 16 hours) greater degree in PTX animals (Table I).

5.) Concentration of sialic acid in gastric mucosa increased in rats receiving PTE. This increase was delayed with respect to the rise in serum glycoproteins and became apparent only at 30 hours. At this time mucosal concentration of sialic acid was significantly greater in PTX rats than in those of Group II (Table I).

6.) With respect to microscopic renal calcification and amounts of neutral mucopolysaccharide in renal parenchyma, results were extremely consistent among animals of each group. No calcification was visible in any

animals at 8 hours. Seventy-five per cent of animals in Group I had microscopic renal calcification, mainly in the tubules, at 16 hours. At 30 hours the degree of calcification had increased severely and was present in 96% of the rats. In none of the animals of Group II was there any calcification visible at 16 hours and only 22% had minimal calcification present at 30 hours. The amount of PAS positive material in the renal parenchyma of the rats of Group I was greatly increased over the normal. This increase was apparent only at 16 hours and was very severe at 30 hours. The increased deposition of glycoprotein involved mainly the cells of the proximal convoluted tubule and the collecting tubules with formation of mucoprotein casts plugging the lumen. Even at 30 hours, rats of Group II had only minimal increase of PAS positive material over the normal. Thus PTE-induced renal deposition of glycoprotein was greatly enhanced by PTX.

With the exception of serum Ca^{++}, values measured were not altered by parathyroidectomy alone when compared to those of untreated, sham-operated rats (Group C at 30 hours).

Discussion. With respect to the enhancement of the calcium-mobilizing property of PTE by parathyroidectomy, the data at 8 and 16 hours confirm earlier studies(1). However, effects at 30 hours are in apparent contradiction with these studies since they show an actual lowering of serum Ca^{++} by comparison with intact rats receiving PTE. But the contradiction is only apparent, since at this stage PTX rats also had widespread extra-osseous calcification which in all likelihood led to lowering of serum Ca^{++}. There was also a definite enhancement by PTX of the glycoprotein-elevating action of PTE. This was reflected not only by greater serum protein-bound hexose and sialic acid levels but also by increased amounts of glycoprotein in the renal parenchyma and in the gastric mucosa by comparison with intact rats given the same doses of PTE. The enhancement of calcium-mobilizing action of PTE by parathyroidectomy has been attributed(1) to removal of Copp's calcitonin(10), a blood Ca^{++} lowering hormone presumably elaborated by the parathyroid (although recent studies suggest that such a calcium-lowering substance might come from the thyroid gland (11)). The finding that parathyroidectomy enhances both the Ca^{++} and the glycoprotein-mobilizing properties of PTE suggests that these share a common biochemical mechanism. However, the data do not clarify the origin of the elevated serum glycoproteins following PTE administration to rats. The drop in serum Ca^{++} in PTX rats after an initial rapid rise can be attributed to earlier metastatic calcification. In turn, it is conceivable that the increased amounts of mucopolysaccharides in tissues could accelerate Ca^{++} deposition because of the affinity of these long chain polyanions for Ca^{++}.

Summary. The effects of PTE on serum Ca^{++} and serum total glycoprotein levels as well as on serum sialic acid levels in normal and parathyroidectomized rats were studied. Alterations in serum glycoprotein and serum protein fractions under these conditions were measured also. The degree of metastatic calcification and of tissue concentrations of glycoproteins was measured by special stains of kidney sections and by measurement of sialic acid concentration in gastric mucosa. The rise in serum Ca^{++} and glycoprotein levels normally occurring in rats given PTE was greatly enhanced by parathyroidectomy. PTE-induced alterations in serum protein and glycoprotein fractions were greater in parathyroidectomized rats. The latter also had more rapid onset of renal calcification and greater tissue deposition of glycoproteins.

1. Stoerk, H. C., Celozzi, E., Proc. Soc. Exp. Biol. and Med., 1963, v114, 714.

2. Munson, P. L., in The Parathyroids, 1961, Thomas, Springfield, Ill.

3. Bradford, R. H., Howard, R. P., Joel, W., Shetler, M. R., A.M.A. Arch. Path., 1960, v69, 382.

4. Menguy, R., Masters, Y. F., Clin. Res., 1964, v12, 275.

5. Engel, M. B., Arch. Path., 1952, v53, 339.

6. Weischelbaum, T. E., Am. J. Clin. Path., 1946, v10, 40.

7. Shetlar, M. R., Foster, J. V., Everett, M. R., Proc. Soc. Exp. Biol. and Med., 1948, v67, 125.

8. Warren, L., J. Biol. Chem., 1959, v234, 1971.

9. Zak, B., Hindman, W. M., Fisher, M., Am. J. Clin. Path., 1956, v26, 1081.

10. Copp, D. H., Cameron, E. C., Cheney, B. A., Davidson, A. G. F., Henze, K. G., Endocrinology, 1962, v70, 638.

11. Foster, G. V., Boghdiantz, A., Kumar, M. A., Slack, E., Soliman, H. A., MacIntyre, I., Nature, 1964, v202, 1303.

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On the Suggested Ionoregulatory Role of the Teleost Caudal Neurosecretory System.* (30055)

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Since the first attempts to uncover the function in fishes of the caudal neurosecretory system and of its neurohemal organ, the urophysis, the notion has prevailed that this system is concerned with osmo(iono)-regulation(1,2). This notion has been substantiated by 3 lines of evidence in recent years: the finding of differences in serum chloride levels in freshwater teleosts subjected to deleterious concentrations of NaCl, in the presence and in the absence of the urophysis(3); the demonstration that urophysial extracts increase the Na content of goldfish by augmenting Na influx across the gill and also by effects at the renal level(4,5); and the electrophysiologic data indicating differential responses of neurosecretory neurons in the caudal system to variations in NaCl level in the blood(6-8).

In contradistinction to this evidence has been the general knowledge that urophysectomized *Tilapia mossambica* (a freshwater euryhaline teleost) can withstand not only their original freshwater environment, but can also be moved into sea water without significant mortality (Bern, unpublished; Reid, unpublished; Fleming and Stanley, unpublished). Similarly *Fundulus kansae* (Fleming and Stanley, unpublished) and *Jenynsia lineata* (Garcia Romeu, personal communication) can also survive after removal of the urophysial region. In addition, acetone-dried extracts of the caudal neurosecretory region in *Tilapia* have revealed no evidence of neurohypophysis-like activity and at best a slight atypical antidiuretic action in rats(9). Even the latter activity was essentially absent from urophysial extracts of the marine teleost, *Chaetodon miliaris* (Sawyer and Bern, unpublished; cf. 5).

In the present paper, we have pooled the data from 2 laboratories (Berkeley, Calif. and Columbia, Mo.) where experimentation has been conducted on the caudal neurosecretory system, in order to record the nature of other evidence not indicative of an osmoregulatory influence of the urophysis and its products. It seems incorrect to us to publish only the data that support a urophysial contribution to osmoregulation. The present negative data need to be considered in assessing the role of this elusive neuroendocrine apparatus.

Materials and methods. In the series of experiments performed in Berkeley, *Tilapia mossambica* weighing between 22 and 56 g were separated into 3 groups: intact, sham-operated and urophysectomized. The surgical technics have been described(3). These 3 categories of *Tilapia* were again subjected to one of the following procedures: (1) anus and urogenital orifice were ligated with a pursestring suture to prevent elimination of feces and urine; (2) a polyethylene tube attached to a small bag was sutured in place in the urogenital orifice to prevent entry of urine into aquarium water; or (3) no further operation was performed. At the beginning of the experiment all these fish were injected intraperitoneally with a salt load (1 ml per 100 g body weight of a solution containing 1238 mEq NaCl, 2.7 mEq KCl, 1.8 mEq

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