by preponderant reabsorption. The results of the present study are consistent with this interpretation. It is shown that in the Dalmatian, under conditions of net tubular secretion of uric acid, the $U_{urate}V/F_{urate}$ ratios do not decline when the GFR and F_{urate} are reduced up to 50%, and that similarly, in the mongrel dog, under conditions of net tubular reabsorption of uric acid, the $U_{urate}V/F_{urate}$ ratios are but little affected. Moreover, in the mongrel as in the Dalmatian, the relationship of excreted to filtered uric acid over a wide range of filtered uric acid loads more closely resembles the response of potassium, which is subject to tubular secretion, than of sodium.

Summary. Reduction of mean renal arterial blood pressure of Dalmatian and mongrel dogs by inflating a balloon-tipped catheter inserted into the aorta just above the renal arteries resulted in a proportionate decrease in GFR, less decrease in C_{PAH}, a precipitous fall in $U_{Na}V/F_{Na}$, no appreciable change in U_KV/F_K. In the Dalmatian, under conditions of net secretion of urate, $U_{urate}V/F_{urate}$ was unchanged or rose somewhat. A comparable response of $U_{urate}V/F_{urate}$ was obtained in mongrels under conditions of net reabsorption of urate, a result consistent with tubular secretion of urate masked by preponderant tubular reabsorption.

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Immunochemical Studies of Bovine Parathyroid Hormone.* (28831)

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Parathyroid hormone (PTH) is apparently rather weakly antigenic in that the few reported attempts to produce antibodies to it have been only partially successful(1,2,3). However, Tashjian *et al*(3) have recently produced antibodies to bovine PTH, as judged by inhibition of biologic activity and by complement fixation analysis. The present report describes preliminary studies of a) the antigenicity of PTH and b) characterization of the PTH-antiPTH system by several immunochemical methods. Because of the great difficulty in obtaining adequate human parathyroid tissue for hormone extraction, the studies thus far have been carried out with bovine PTH.

Materials and methods. Parathyroid hormone preparation. Starting with fresh frozen bovine parathyroid glands (PTG), 4 different hormonal extracts were prepared: (a) an aqueous extract (A-PTE) prepared by homogenizing fresh frozen PTG in an equal quantity of distilled water, centrifuging, and obtaining the supernatant, (b) an ether precipitate (EP-PTE) (biologic activity 40 U.S.P. units/mg) (4), and (c) a partially purified trichloroacetic acid precipitate (PP-

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PTE) (biologic activity 425-475 U.S.P. units/mg) prepared by the method of Aurbach(5), and (d) a highly purified extract (HP-PTE) prepared by further purification of the PP-PTE by gel filtration(1). Aqueous extracts of human PTG and of non-parathyroid tissue were prepared as in (a) above.

Antiserum preparation. Four male albino rabbits weighing 2.5-3.5 kg were immunized with PP-PTE dissolved in 0.005 M acetic acid and emulsified in complete Freund's adjuvant. Two of the rabbits received initial doses of 0.25 mg PP-PTE by foot pad, then biweekly I.M. injections of 0.1 mg for 2 months, and then 0.2 mg monthly for a total dose of 1.5 mg per rabbit in 6 months. The other 2 rabbits received large initial injections of 25 mg PP-PTE by multiple foot pad and I.M injections, followed in one month by 10 mg, and then 2 to 5 mg monthly for a total dose of 51 mg per rabbit in 6 months. Starting one month after initial injection, the rabbits were bled weekly by ear artery and the sera were analyzed for precipitins to PTE. Those sera revealing precipitins were stored frozen without preservative. The antiserum used in the subsequent immunochemical studies was from a pool composed of serum obtained at different times from one rabbit. Non-parathyroid antibodies were absorbed from the antiserum by (a) adding 1/5 volume of a 1:1 mixture of beef serum and an aqueous extract of beef thyroid tissue in serial aliquots, incubating at 37°C for 2 hours, centrifuging, and retaining the supernatant, or (b) pretreatment of the agar slides by filling the antiserum wells with the beef serum and thyroid extract mixture and allowing diffusion to occur before adding the reactants(6).

Immunochemical methods utilized. A) Microimmunodiffusion(7). B) Quantitative microimmunodiffusion. A trough filled with absorbed antiserum and a parallel row of circular wells filled with serial dilutions of PP-PTE were arranged longitudinally on the agar-covered glass slide and allowed to react. The highest antigen dilution developing a visible precipitin line was considered to be the titer of the antiserum. C) Microimmunodiffusion inhibition. After determining the antiserum titer as above, the procedure was repeated, but with pre-treatment of the slide by filling the antiserum trough with a known antigen or unknown substance and allowing diffusion to occur. The antiserum and antigen were then added as in (B) above and the degree of inhibition of the initial reaction observed. D) Microimmunodiffusion(7) with antigen enrichment(8). A trough filled with antiserum, a similar trough filled with a known PP-PTE, and a row of wells between (but nearer the antigen trough) were arranged longitudinally on the slide. The wells were filled with various PTE or unknown preparations to determine the degree of similarity with the known antigen and also the degree of enhancement of the precipitin line by the preparations added to the wells. E) Immunoelectrophoresis(9) with modifications of the electrophoretic procedure(10). F) Hemagglutination and hemagglutination inhibition, using a modification of the technic of Boyden(11). G) Inhibition of biologic activity. Equal amounts of PP-PTE were added to normal rabbit serum (NRS) and to rabbit anti-PTH serum (RAPS) to a concentration of 20 U PTE/ml serum. These sera were incubated at 4°C overnight, centrifuged and the supernatant sera bioassayed by the technic of Munson(4).

Results. By the microimmunodiffusion technic, precipitins to PP-PTE were observed in serum from each rabbit within 90 to 120 days after initiating immunization. The intensity of the precipitin line varied among the rabbits but was not related to the dosage schedule used. The unabsorbed antisera revealed multiple precipitin lines (Fig. 1a), the nonspecific reactions being due to components of beef serum and thyroid gland. After absorption of the antiserum with beef serum and beef thyroid gland extract, the PP-PTE-antiserum reaction revealed a single precipitin line (Fig. 1b). Absorbed antiserum was therefore used for all following studies.

When tested by the quantitative immunodiffusion technic, a precipitin line was detectable at a PP-PTE dilution containing 1.5 μg protein(12) and at a HP-PTE dilution containing 0.015 μg protein. By immunodiffusion with antigen enrichment, several differ-

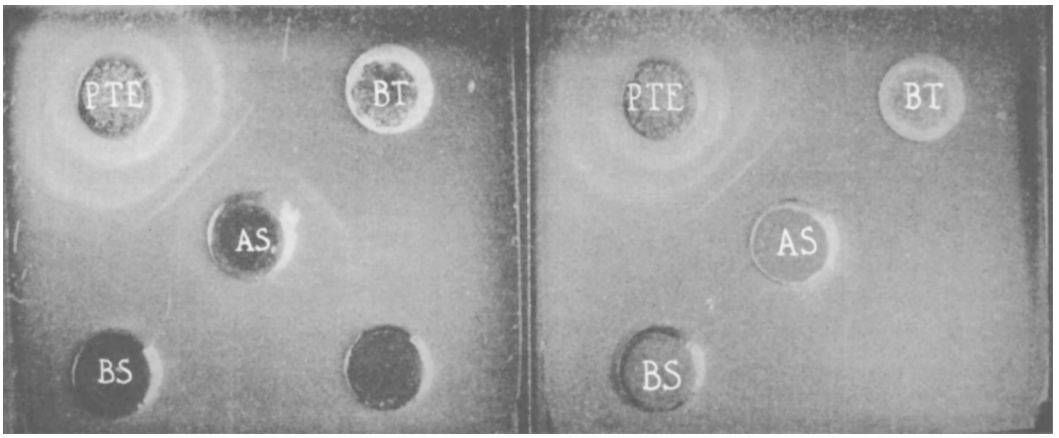

FIG. 1a (left). Immunodiffusion pattern with unabsorbed anti-PTH serum (AS), showing reaction with PTE, beef serum (BS), and beef thyroid extract (BT). Fig. 1b (right). Immunodiffusion pattern after absorption of antiserum, showing single precipitin line with PTE and no reaction with beef serum or thyroid extract. (The halos surrounding the PTE well represent precipitation of nonspecific PP-PTE proteins in barbital buffered agar gel.)

ent preparations of PP-PTE and EP-PTE (and also a highly purified preparation obtained from Dr. Harold Rasmussen), all revealed a reaction of identity with enhancement of the precipitin line. These same preparations which caused enhancement also caused inhibition of precipitin line formation by the immunodiffusion inhibition technic,

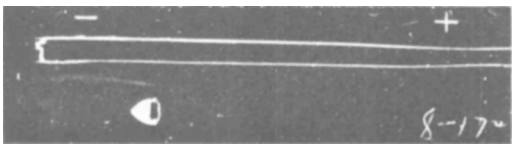

FIG. 2. Immunoelectrophoretic pattern of reaction between PTE and absorbed antiserum, showing single arc in cathodic area (Barbital Buffer, pH 8.8).

the degree of inhibition correlating with the expected hormonal potency of the preparations. An A-PTE preparation also revealed inhibition. Aqueous extracts of human parathyroid adenomas removed surgically and human parathyroid glands removed within 3 hours after death revealed no immunologic activity by any of the above methods.

Immunoelectrophoretic studies revealed that PTH migrated with a cathodic mobility and formed a single arc (Fig. 2).

Preliminary hemagglutination studies revealed an antiserum titer of 1:128 after absorption of the antiserum. Hemagglutination inhibition was caused by the same preparations, and in the same semi-quantitative relationship as in the immunodiffusion inhibition studies.

In 2 separate experiments using a different PP-PTE preparation each time, rabbit anti-PTH serum (RAPS) completely inhibited the biological activity of PP-PTE (Table I).

Discussion. The present studies indicate that PTH is antigenic, the induced antibody titer being more dependent upon duration of immunization than upon magnitude of antigen dose. It was expected that the antiserum

TABLE I. Comparative Effect of Normal Rabbit Serum (NRS) and Rabbit Anti-PTH Serum (RAPS) on Biologic Activity of PTE in Rats.

	Exp I		Exp II	
Treatment	No. rats	Serum calcium, mg/100 ml	No. rats	Serum calcium, mg/100 ml
NRS	5	6.68 ± .08*	5	7.04 ± .36*
NRS + PTE	5	7.66 ± .25 (P<.01)	6	8.98 ± .19 (P<.001)
RAPS + PTE	6	6.46 ± .09	6	7.35 ± .12

* Mean ± S.E.

would be heterogeneous, because the antigen was only partially purified PTE. Absorption of the antiserum with beef serum and thyroid extract apparently removed non-specific antibodies, as the absorbed antiserum revealed a single immunodiffusion precipitin line and a single immunoelectrophoretic arc when reacted with the antigen. PTE preparations, varying from very crude to highly purified, all a) enhanced the precipitin reaction of a known PP-PTE and b) inhibited the subsequent anti-PTE potency of the antiserum when mixed with it, indicating a common antigen in these preparations. It is concluded that this antigen is PTH and not an artifact introduced during processing of the PTG, nor one which failed to be removed during final purification. The antiserum exerted a specific anti-hormone effect on the biologic calcium mobilizing activity of bovine parathyroid hormone in rats. The increase in antigenic potency with progressive purification of the PTE preparations further indicates that the PTH-antiPTH serum reaction is immunologically specific for parathyroid hormone.

The inability to demonstrate immunologic activity in aqueous extracts of human parathyroid tissue may be due to insufficient human tissue available for extraction or to immunologic species specificity of the hormone. The fact that aqueous extracts of an equal weight of bovine parathyroid tissue did reveal immunologic activity by immunodiffusion inhibition suggests species specificity as the more likely explanation.

Summary. An antiserum to bovine parathyroid hormone has been produced in each of 4 rabbits studied, indicating that this hormone is antigenic. The absorbed antiserum induced a single precipitin line by immunodiffusion and immunoelectrophoresis with PTH of variable states of purity, and exerted a specific anti-PTH effect on biologic activity. There are apparent antigenic differences between beef and human parathyroid hormone.

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Effect of Thyroxine on Thyroid Uptake of Thiourea-S35.* (28832)

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Previous studies in guinea pigs revealed that thyrotropin (TSH) was capable of accelerating the intrathyroid metabolism of thiourea-S35 as measured by the formation of sulfate-S35, this being the major metabolite of oxidative desulfuration of the labeled antithyroid compound(1). However, in the same experiment, administration of potas-

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