A Radioimmunoassay for Parathyroid Hormone in Man* (33050)

ERIC REISS AND JANET M. CANTERBURY

Department of Medicine, Michael Reese Hospital and Medical Center, Chicago, Illinois 60616

The development of highly sensitive radioimmunoassay techniques for measuring peptide hormones in human sera has profoundly influenced current concepts in clinical endocrinology (1,2). The assay for parathyroid hormone (PTH), first described in 1963 (3), has made possible important studies of parathyroid physiology in species other than man (4,5). Investigations in man have been hampered by the unavailability of highly purified human PTH and by difficulties in producing antisera suitable for radioimmunoassay. In the only human studies reported, Berson and Yalow (6,7) noted a considerable overlap of PTH concentration between normal and hyperparathyroid sera; 14 of 29 plasmas from patients with surgically confirmed hyperparathyroidism yielded results within the normal range. In this report, we describe the development of an antiserum to bovine PTH that possesses high affinity for PTH in human sera and yields unequivocal separation of normal, hypoparathyroid, and hyperparathyroid sera.

Materials and Methods. Partially purified beef PTH was prepared from crude parathyroid gland powder (Wilson Laboratory) by the method of Rasmussen (8). This preparation, which was used for all immunizations, had a biologic activity of 150–200 units/mg.

Antibody production was attempted in five species: guinea pig, rabbit, goat, horse, and chicken. Many animals were injected by many different routes and time schedules, with and without adjuvant. Only one chicken gave an antiserum suitable for radioimmunoassay. This animal was a cockerel, 8 weeks old at the time of the first injection. The antigen was dissolved in saline and always given without adjuvant. Two intravenous injections of 40 mg each were given 4 weeks apart. One week after the second dose, 10 mg was injected intramuscularly on each of 4 consecutive days. Thereafter, 20 mg was injected intramuscularly at monthly intervals. The antibody was detected in maximal concentration 5 months after the first injection.

Highly purified bovine hormone was prepared by the methods of Rasmussen (8) and of Aurbach and Potts (9). The biologically active peaks were cycled a second time to afford better separation. The final product gave a single band on starch gel electrophoresis and was assayed at a biologic potency of 1800–2000 units/mg. This preparation was labeled with ^{131}iodine by the procedure of Greenwood and Hunter (10), and the iodinated hormone (PTH*) was removed from the reaction mixture by absorption to silica gel (11). On electrophoresis, 92–99% of the radioactive PTH* thus prepared adhered to the point of application.

All dilutions of antisera, test sera, PTH*, and unlabeled PTH were made in barbital buffer, pH 8.6, ionic strength 0.05, containing 1% bovine serum albumin. One-tenth ml of a 1:1000 dilution of antiserum was mixed with an equal volume of buffer containing various quantities of test sera or unlabeled highly purified bovine PTH and incubated at 4°C for 96 hours (4). One-tenth ml of PTH* was then added, and the incubation was continued for 48 hours. The final dilution of antiserum was 1:3000. One-tenth ml of a 1:1000 dilution of normal chicken serum was incubated with PTH* as a control.

Separation of protein-bound from free hormone was accomplished by electrophoresis on cellulose acetate, 34 V/cm for 12 min at room temperature in an EDTA–Tris–borate buffer, pH 8.6. This procedure gave a reproducible 2-cm distance between bound and free hormone as indicated by scanning of the radioactivity of many strips. The radioactivity adhering to the point of application was interpreted as representing free hormone. The electrophoretic strips were cut into segments containing free and protein-bound hor-

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mone and counted in an autogamma counter for a sufficient period to yield a counting error of less than 1%.

With this procedure, 3–12% of the PTH* migrated electrophoretically with the serum proteins of normal chicken sera. This probably represented incubation damage and incomplete purification of PTH* after the labeling procedure. Addition of up to 20 μl of hypoparathyroid serum to the mixtures did not appreciably increase the fraction of incubation damage. (For the assays of circulating PTH the incubation mixtures never contained more than 10 μl of test serum.) For each experiment the percentage of total radioactivity in the protein fraction of normal chicken sera controls was determined and subtracted from the protein-bound radioactivity of test samples.

Results. Since human PTH of sufficient purity to serve as a reference standard has not yet been prepared, we followed the procedure of Berson and Yalow (6) of relating the potency of all test sera to an arbitrarily selected hyperparathyroid serum derived from a uremic subject with severe secondary hyperparathyroidism. This was assigned a concentration of 1000 μl-equivalents/ml. When results were expressed as a percentage of the bound to free (B/F) ratio of PTH* in tubes containing no unlabeled hormone, the standard curve was remarkably reproducible (Fig. 1).

All determinations were performed on serum, which was rapidly separated from the clot and stored in the frozen state. Plasma collected with heparin yielded erratic and uninterpretable results (Table I).

All sera were assayed in duplicate at two widely differing concentrations. The coefficient of variation of replicates at different concentrations was 8% for sera containing normal or high concentrations of hormone.

Twenty-nine healthy adults had a PTH concentration of 28 ± 13 (mean ± 1 SD) (Fig. 2). In four children with clinically severe idiopathic hypoparathyroidism and in one adult with severe postoperative hypoparathyriodism, PTH concentration was unmeasureably low.

The sera of 32 patients with surgically proved primary hyperparathyroidism were measured. All except one yielded results that are clearly above the normal range. The first serum from this patient gave a concentration of 56; a second specimen collected 2 months

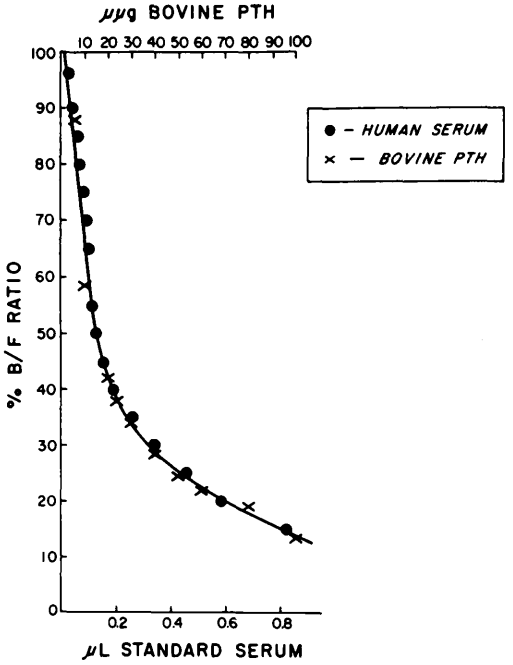

FIG. 1. Function relating percentage of B/F ratio to the concentration of purified bovine PTH and standard human hyperparathyroid serum. The quantities on the abscissa refer to absolute amounts in the incubation mixture. The curves through the two sets of points appear to be identical (final dilution of antiserum, 1:3000).

TABLE I. Examples of Interference of Heparin (143 units/5 ml of blood) with PTH Assay.[a]

	PTH (μl-equivalents/ml)			
	Serum		Plasma (heparin)	
Patient	(1)	(2)	(1)	(2)
Van.	450	480	250	170
H.B.	450	442	400	170
A.K.	1000	1030	405	110

[a] Samples with and without heparin were collected at the same time. Columns (1) and (2) refer to 0.2 μl and 0.5 μl of test sera in incubation mixture respectively.

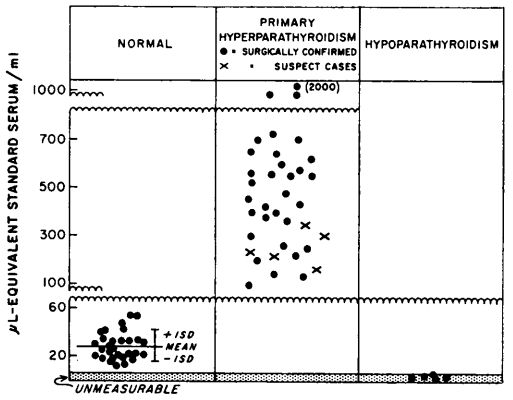

FIG. 2. Each point represents the mean of four separate determinations. We have arbitrarily accepted a B/F depression of 4% or less as being too small for precise measurement and refer to such samples as unmeasureable.

later was measured at 148. High PTH levels were measured in five patients in whom primary hyperparathyroidism was highly likely on clinical grounds by the usual criteria of diagnosis. All had mild to moderate hypercalcemia for which no other cause could be found on careful clinical evaluation.

Discussion. Clinical and physiologic studies of PTH in man have been hampered by difficulties with the radioimmunoassay. The assay depends on the cross-reactivity between antisera produced against bovine PTH and PTH in human sera.

The antiserum produced in the chicken has a high affinity for bovine PTH, as indicated by the steep slope of the B/F ratio as a function of standard hormone concentration. Five to 10 $\mu\mu$g of bovine PTH can be detected in the assay. How completely this antiserum reacts with human PTH cannot be definitely determined without highly purified human PTH, which has not yet been prepared. However, the data in Fig. 1 suggest that cross-reactivity with PTH in human sera is good, and it is certainly adequate for the measurement of PTH concentration in normal and abnormal human sera.

In this initial evaluation of the method, we have restricted ourselves to well-defined clinical problems. The excellent clinicopathologic correlation observed is encouraging. The specificity of the assay has been further substantiated by depression of normal PTH concentrations in response to intravenous infusions of calcium and by measurement of the sequential decline of PTH from high to normal concentrations in patients undergoing excision of parathyroid adenomas.

If complete cross-reactivity between the chicken antiserum to bovine PTH and human PTH is assumed (Fig. 1), the normal concentration of PTH in man approximates $2\text{–}3 \times 10^{-10}$ *M*. This is one order of magnitude lower than estimates from careful bioassays of large quantities of extracted human sera (12) but exceeds Berson's (7) estimates by one order of magnitude. These differences will be resolved only after highly purified human PTH has been prepared. For the present, the lack of an absolute standard poses difficulties only in comparing results between different laboratories. By current standards of evaluation, including sensitivity, reproducibility, and clinicopathologic correlation, the antiserum and procedure here described appear to be satisfactory.

Summary. An antiserum to bovine parathyroid hormone with high affinity for bovine and human parathyroid hormone was produced in a chicken. It made possible sensitive and reproducible measurement of the hormone in normal and abnormal human sera by radioimmunoassay. Unequivocal separation of normal, hypoparathyroid, and hyperparathyroid sera was obtained.

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Further Studies on the Erythrocyte Anti-Inflammatory Assay (33051)

J. H. BROWN AND H. K. MACKEY

Mead Johnson Research Center, Evansville, Indiana 47721

Similarities between the action of agents and procedures on red cells and lysosomes suggested that membranes bounding erythrocytes and lysosomes have common properties. Work based on this assumption has shown that nonsteroidal anti-inflammatory agents such as phenylbutazone and indomethacin protect erythrocytes from heat-induced hemolysis (1).

In our search for a suitable solvent to test steroids in this system, the effect of various concentrations of dimethylsulfoxide (DMSO) and ethyl alcohol on heat-induced hemolysis of erythrocytes was determined. The results of these studies are summarized in this report.

Materials and Methods. The *in vitro* system for studying the action of nonsteroidal anti-inflammatory agents has been described previously (1). The same methods were utilized in these studies except for solubilization of steroids or other drugs in 0.067 *M* sodium phosphate buffer, pH 7.4, containing 0.9% saline and either 1.0% DMSO or 5.0% ethanol.

The following steroids, listed in subsequent tables by generic or common names, were employed in studies of the present report: cholanic acid; cholesterol, 5-cholesten-3β-ol; cholic acid, 5β-cholanic acid-3α,7α,12α-triol; cortisone, 4-pregnen-17α,21-diol-3,11,20-trione; dexamethasone,1,4-pregnadien-9-fluoro-16α-methyl-11β,17α,21-triol-3,20-dione; diethylstilbestrol, [3,4-bis-(-4-hydroxyphenyl)-3-hexane]; dimethisterone, 6α-methyl-17α-propynylandrost-4-en-17β-ol-3-one monohydrate; ergosterol, 5,7,22-cholestatrien-24β-methyl-3β-ol; estradiol, 1,3,5(10)-estratrien-3,17β-diol; 17α-ethinylestradiol, 1,3,5(10)-estratrien-17α-ethinyl-3,17β-diol; etiocholanolone, 5β-androstan-3α-ol-17-one; hydrocortisone, 4-pregnen-11β,17α,21-triol-3,20-dione; medroxyprogesterone, 17α-hydoxy-6α-methyl-4-pregnen-3,20-dione acetate; methyltestosterone, 4-androsten-17α-methyl-17β-ol-3-one; paramethasone acetate, 6α-fluoro-11β,17,21-trihydroxy-16α-methyl-pregna-1,4-diene-3,20-

TABLE I. Action of DMSO and Ethanol on Heat-Induced Hemolysis.

DMSO (%) in buffered saline, pH 7.4[a]	Inhibition (%)
1	7.8
5	51.6
10	70.8
20	46.6
Above 20	Discoloration and precipitation occurred
Ethanol (%) in buffered saline, pH 7.4[a]	
1	No apparent effect
5	No apparent effect
10	Hemolysis enhanced with discoloration

[a] Assays were in duplicate.