JCI The Journal of Clinical Investigation

Hormone synthesis and secretion by rat parathyroid glands in tissue culture

William Y. W. Au, … , Paula H. Stern, Lawrence G. Raisz

J Clin Invest. 1970;49(9):1639-1646. https://doi.org/10.1172/JCI106381.

Research Article

Rat parathyroid glands maintained in organ culture secrete biologically active parathyroid hormone (PTH) and synthesize and secrete labeled proteins from ^{3}H- or ^{14}C-labeled amino acids added to the medium. The amounts of biological activity and labeled protein in the medium are both inversely proportional to the calcium concentration. Some of the labeled low molecular weight protein was identified as PTH which had been synthesized and secreted in culture by preliminary isolation on Sephadex G-100 columns and further purification using an antibody to bovine PTH which cross-reacted with rat PTH. The cross-reacting antibody inhibited the biological effects of rat PTH and caused hypocalcemia in intact rats. The antibody bound some of the labeled low molecular weight protein of the medium at neutral pH so that it migrated as a large molecular weight complex on Sephadex. Biologically active, labeled PTH was recovered by dissociation of this complex in acid and rechromatography.

Hormone Synthesis and Secretion by Rat Parathyroid Glands in Tissue Culture

WILLIAM Y. W. AU, ALAN P. POLAND, PAULA H. STERN, and LAWRENCE G. RAISZ

From the Departments of Pharmacology and Toxicology and of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620

ABSTRACT Rat parathyroid glands maintained in organ culture secrete biologically active parathyroid hormone (PTH) and synthesize and secrete labeled proteins from ^{3}H- or ^{14}C-labeled amino acids added to the medium. The amounts of biological activity and labeled protein in the medium are both inversely proportional to the calcium concentration. Some of the labeled low molecular weight protein was identified as PTH which had been synthesized and secreted in culture by preliminary isolation on Sephadex G-100 columns and further purification using an antibody to bovine PTH which cross-reacted with rat PTH. The cross-reacting antibody inhibited the biological effects of rat PTH and caused hypocalcemia in intact rats. The antibody bound some of the labeled low molecular weight protein of the medium at neutral pH so that it migrated as a large molecular weight complex on Sephadex. Biologically active, labeled PTH was recovered by dissociation of this complex in acid and rechromatography.

INTRODUCTION

The proportional control of parathyroid hormone (PTH) secretion by the serum calcium concentration has now been demonstrated both in vivo and in vitro using both bioassay and immunoassay (2–6). There is also an inverse relationship between calcium concentration and amino acid uptake by the parathyroids (5, 7). The present studies are an extension of earlier work (4, 5) on the relationship between calcium concentration and the synthesis and secretion of hormone by rat parathyroid glands in organ culture. Using radioactive amino acids as precursors, the secretion of labeled low molecular weight proteins was measured and related to PTH by bioassay of the medium. Bioassay was based on measurements of release of previously incorporated ^{45}Ca in cultures of fetal rat bones (8). Antibodies to PTH were obtained by injecting bovine parathyroid hormone (bPTH) into guinea pigs. Some of the antisera cross-reacted with rat parathyroid hormone (rPTH) sufficiently to produce hypocalcemia when injected into rats in vivo and to inhibit bone resorption stimulated by rPTH as well as bPTH in tissue culture. Using this antibody, a chromatographic method was developed for isolation of rPTH which had been labeled by incorporation of radioactive amino acids in tissue culture.

METHODS

Parathyroid gland culture. Parathyroid glands were obtained from adult male rats, freed from thyroid and adherent connective tissue under a dissecting microscope, and cultured on small pieces of Millipore filter on metal screens in vessels containing 0.5 ml of medium in an atmosphere of 5% CO_2–95% O_2. The medium was Basal Medium Eagle, with 5 or 50% serum (heat-inactivated at 60°C for 30 min) from thyroparathyroidectomized rats. The medium was made up with varying calcium concentrations, and with part or all of the amino acids of Eagle's medium replaced by a mixture of ^{3}H- or ^{14}C-labeled amino acids made up from those amino acids which account for most of the residues of bPTH (or a commercial mixture reconstituted from algal protein hydrolysate, New England Nuclear). The final concentration of radioactivity was 1 μCi/ml for ^{14}C-labeled and 10 μCi/ml for ^{3}H-labeled amino acid mixtures. This represented the addition of approximately 10^{-8}mole/liter and 3×10^{-7}mole/liter of total amino acids to the medium respectively compared to more than 10^{-4}mole/liter contributed by serum at 50% concentration.

Parathyroid hormone bioassay. The bioassay for PTH based on resorption of embryonic bone in tissue culture has been described previously (8). Samples of medium or lyophilized chromatographic fractions from parathyroid gland cultures were diluted either in Eagle's medium with serum or in a chemically defined medium (BGJ, Grand Island Biological

A portion of this work was previously presented in abstract form (1).

Dr. Poland's present address is The Rockefeller University, New York. Dr. Stern's present address is Northwestern University School of Medicine, Chicago, Ill.

Received for publication 12 March 1970.

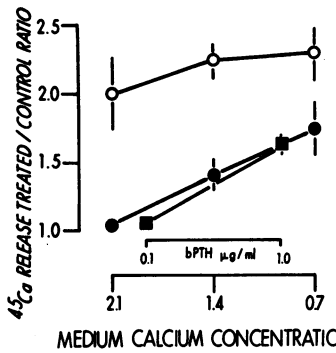


FIGURE 1 Secretion of rPTH in tissue culture. Four parathyroid glands were cultured per ml of medium (or two per culture vessel) at the indicated calcium concentrations for 2 days. The gland culture medium was then diluted with BGJ-albumin to a concentration of 0.5 glands/ml (—○—) or 0.1 glands/ml (—●—) and its ability to stimulate bone resorption in tissue culture compared with bPTH (—■—). Values are mean ±SE for the treated/control ratio of ^{45}Ca release for four pairs of cultures.

Co.) with 1 mg/ml bovine serum albumin fraction V (Pentex) which was found to support bone resorption in tissue culture (9). The calcium concentration of the diluting medium was adjusted to compensate for differences in the calcium concentration of parathyroid culture medium. Bone resorbing activity was measured as the release of previously incorporated ^{45}Ca from embryonic fetal bone compared to untreated paired controls (T/C ratio). The amount of rPTH was assessed by comparison with the effects of purified bPTH added to similar media.

Chromatography of culture medium. Aliquots of parathyroid gland culture medium or control medium (which had been incubated without parathyroid glands) were chromatographed on columns of Sephadex G-100 using ammonium acetate buffer (0.2 M NH_4OAc, pH 4.7). Chromatography was carried out at 4°C. Fractions were assayed for protein content by UV absorption and for radioactivity in a liquid scintillation counter. For bioassay the appropriate fractions were pooled and lyophilized, redissolved in 0.01 N acetic acid, and relyophilized. Some of this material was also rechromatographed on Sephadex G-25 or used for isolation with antiserum as indicated below. In some experiments the amount of radioactive protein recovered from the column was compared with the amount obtained by precipitating the proteins from the medium with 5% trichloroacetic acid (TCA).

Antibodies to bPTH. Guinea pigs were immunized with either partially purified bovine parathyroid TCA powder or purified bPTH (2000 U/mg [10], the purified material used in initial experiments was kindly provided by Dr. Gerald Aurbach). The material was mixed with complete Freund's adjuvant (Difco) and injected either into the footpad or intracutaneously every 2–4 wk for at least 4 months (11). Sera were then obtained by heart puncture and screened for precipitating antibody to bPTH by the double-diffusion method of Ouchterlony. Animals with precipitins in their serum were reinjected with antigen and rebled 2–4 wk later. Subsequent sera were tested for their ability to block bPTH or rPTH in tissue culture or for their effect on serum calcium concentration after intravenous injection in the intact rat.

Antibody isolation of labeled rPTH. Biologically active, low molecular weight protein fractions isolated by Sephadex chromatography from parathyroid culture media were incubated with anti-bPTH guinea pig serum which had previously been shown to react with rPTH by one of the methods described above. Isolation of the antigen-antibody complex by chromatography and dissociation of rPTH by rechromatography in acid pH is described under Results.

RESULTS

The inverse relationship between the secretion of biologically active rPTH in culture and calcium concentration of the medium is illustrated in Fig. 1. Biological activity could be detected in the medium even at a high calcium concentration when four parathyroid glands were cultured per milliliter. The amount of rPTH secreted in low calcium medium was remarkably high, representing the equivalent of about 10 µg of bPTH secreted per rat parathyroid gland per 48-hr culture. The dry weight of these glands was from 20 to 50 µg.

TABLE I

Effect of Changing Calcium Concentration on Secretion of Labeled Proteins by Rat Parathyroid Glands in Tissue Culture

	Ca concn	Column fractions			Total labeled protein A + B + C	TCA precipitate
		A	B	C		
	mmoles/liter	*cpm/gland*				*cpm/gland*
Control medium	1.4	7,230	8,350	720	16,300	16,000
High Ca culture	2.1	8,060	10,260	3,070	21,390	23,760
Normal Ca culture	1.4	12,250	9,150	7,440	28,840	33,520
Low Ca culture	0.7	21,840	11,870	9,880	43,590	42,435

Values are the sums of the counts per minute for A, B, C fractions similar to those indicated in Fig. 2. The sum of these is compared with the radioactivity in a washed 5% trichloroacetic acid (TCA) precipitate of an aliquot of the same medium before chromatography.

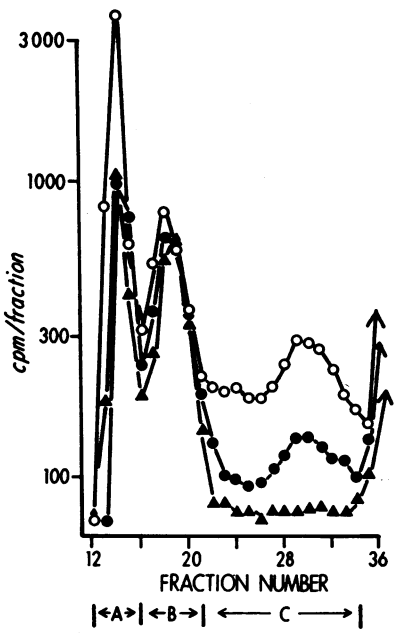


FIGURE 2 Effect of calcium on the secretion of labeled proteins by rat parathyroid glands in tissue culture. Glands were cultured in Eagle's medium with 50% serum and commercial amino acid-^{3}H mixture which was chromatographed on Sephadex G-100 (0.9 × 60 cm column 0.2 M NH_4OAc buffer pH 4.7). 0.5 ml aliquots of control medium which had been incubated without parathyroid glands (—▲—), and 0.5 ml of medium from two parathyroid glands cultured at high calcium (2.1 mmoles/liter, —●—) or low calcium concentrations (0.7 mmole/liter, —○—) are compared. The bioassays in Fig. 1 were obtained from aliquots of the same media. Counts per minute per fraction are shown on a logarithmic scale. Labeled protein represented 1–5% of the total radioactivity in the medium.

When parathyroid glands were incubated with ^{3}H-labeled amino acids in the medium they synthesized and secreted substantial amounts of labeled protein. The amount of labeled protein recovered by chromatography and by precipitaion with TCA was similar (Table I). On Sephadex G-100 columns this labeled protein could be divided into three peaks (Figs. 2 and 3). Fraction A consisted of large molecular weight proteins. Radioactivity in fraction A usually increased in media from parathyroid glands cultured at low calcium concentrations. The B fraction coincided with serum albumin and was labeled to the same extent whether parathyroid glands were present or not; presumably by binding of the labeled amino acids. When the serum concentration of the media was reduced to 5% the radioactivity in the B fraction was also reduced. Neither the A nor B peaks were biologically active (Table II). The C fraction contained small amounts of low molecular weight material, presumably small proteins and peptides. A radioactive peak was regularly observed in the C fraction of medium from glands cultured at low calcium concentration. The relative position of this peak varied somewhat in different experiments. This was due in part to differences in the elution volume of the labeled amino acids themselves on different columns. The amount of radioactivity and biological activity in the peak was inversely proportional to the calcium concentration in the medium. The radioactivity and biological activity in the C fraction of parathyroids cultured at low Ca concentration increased after the first 2 days in culture (Table III). Thereafter high concentrations of radioactivity and biological activity in a sharp peak persisted for 12 days.

In other experiments a difference in radioactivity and biological activity in C fractions from glands cultured in high and low calcium medium persisted for up to 12 days. After this, the results were variable. Large amounts of labeled protein appeared in all fractions (A, B, and C) in some cultures while in others the amount decreased indicating either rapid tissue breakdown or gradual cessation of protein synthesis. As had been noted previously (12), the effects of calcium on synthesis and secretion of labeled proteins were reversible (Table IV). The radioactivity in both A and C fractions was decreased when glands were transferred to medium with a high calcium content and the radioactivity

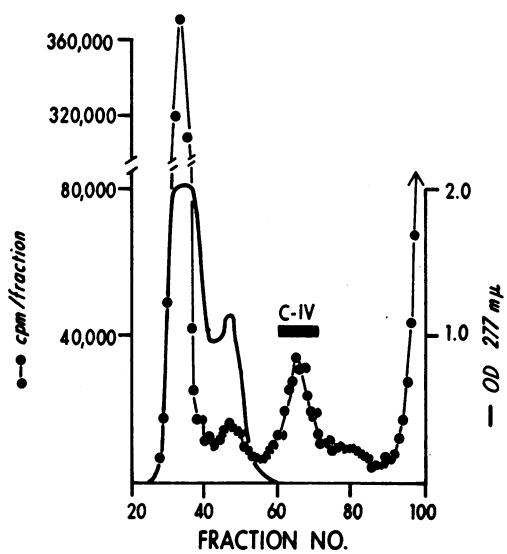


FIGURE 3 Synthesis and secretion of labeled protein by rat parathyroid in culture. Glands were cultured for 12 days in Eagle's medium with 5% serum. Medium was changed every 2 days. 3 ml of medium containing the secretion from 24 parathyroid glands was chromatographed on Sephadex G-100 (2.5 × 90 cm column, 0.2 M NH_4OAc buffer at pH 4.6). This graph shows the results for 6–8 day culture medium and was typical of the results for 2–12 days in culture. The C peak was divided into three fractions. The biological activity was found in the indicated sharp radioactive peak, C-IV.

TABLE II

Bone Resorbing Activity of Labeled Protein Fractions Isolated from Parathyroid Gland Culture Medium

Fraction	High Ca (2.25 mM)		Low Ca (0.75 mM)		Control	
		T/C ratio		T/C ratio		T/C ratio
	μg/ml		*μg/ml*		*μg/ml*	
A	140	1.12 ±0.11	305	1.13 ±0.08	401	1.01 ±0.10
B	142	1.03 ±0.04	238	1.06 ±0.06	170	1.15 ±0.05
C	6	1.08 ±0.03	8	2.76 ±0.35	4	1.18 ±0.04
bPTH			1	2.55 ±0.07		

Medium from glands cultured in Eagle's medium with 5% serum at indicated calcium concentrations and control medium incubated without parathyroid glands were chromatographed and the A, B, and C fractions tested for stimulation of bone resorption at a concentration representing the secretion of one parathyroid gland per ml. Values are mean ±SE for treated/control ratio for ^{45}Ca release for four paired bone cultures. Underlined values significantly different from 1.0, $P < 0.01$.

increased again when the glands were returned to low calcium medium.

A partially purified rPTH material could be obtained when a C fraction pooled from several culture media was rechromatographed on Sephadex G-25. The biological activity was still associated with a peak of radioactivity (Fig. 4). In other experiments such as those illustrated in Fig. 3, a relatively sharp peak of radioactivity was found in the C fraction on Sephadex G-100 which was biologically active while adjacent fractions did not cause significant stimulation of bone resorption. The materials obtained by these two chromatographic methods were used in subsequent antibody isolation studies.

Effects of anti-bPTH sera in tissue culture. Sera from 7 of 14 guinea pigs immunized with crude or purified bPTH showed precipitins against bPTH by the double-diffusion method of Ouchterlony. Several of these sera were tested against a biologically active C

TABLE III

Labeled Protein and Biological Activity in Low Molecular Weight Fraction of Parathyroid Gland Culture Fluid

Fraction	Days in culture	Labeled protein	^{45}Ca release T/C ratio
		cpm/gland	
C-I	0–2	1690	1.50 ±0.20
C-II	2–4	7540	2.44 ±0.25
C-III	4–6	6110	2.27 ±0.20
C-IV	6–8	8540	2.07 ±0.20
C-II-AI	2–4	2400	1.81 ±0.10
bPTH 1 μg/ml			1.92 ±0.19
bPTH 0.1 μg/ml			1.08 ±0.06

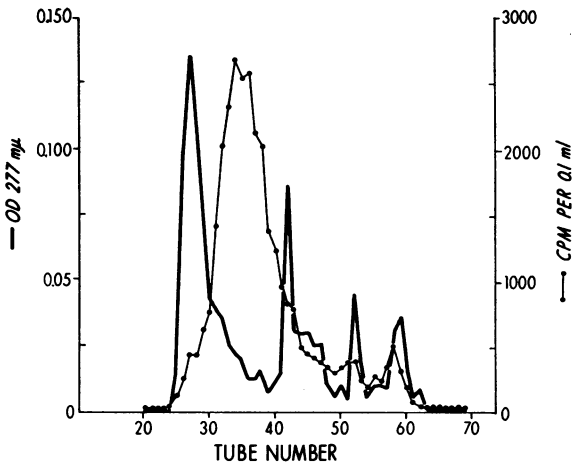
Eight parathyroid glands were cultured per ml of medium in Eagle's medium with 5% serum. A sharp radioactive peak of low molecular weight was isolated (see Fig. 3), lyophilized, and bioassayed at a concentration representing one gland per ml. Bioassay values are mean ±SE for treated/control ratio of ^{45}Ca release of four paired cultures. C-II-AI represents antibody material isolated from the original C-II by the antibody-column method described in the text. Underlined values significantly different from 1 at $P < 0.01$.

TABLE IV

Effect of Changing Medium Calcium Concentration on the Amount of Labeled Protein Released by Rat Parathyroid Glands in Culture

Days	Ca^{++} in media	Radioactivity in medium		
		A	B	C
		cpm/gland		
0–3	Low	16,980	6410	8,490
3–6	Low	33,600	7770	10,531
6–9	Low	36,120	8600	10,980
	High	18,000	6890	3,440
9–12	Low	31,350	7630	10,260
	Low (post high)	44,280	9330	8,490

10 parathyroid glands were cultured per ml of Eagle's medium with 50% serum containing ^{14}C amino acids. Medium was changed every 3 days. For 0–3 and 3–6 days medium from all glands was pooled. Half of the glands were kept in low Ca medium (1.4 mM) thereafter and half were transferred to high Ca (4 mM) for 6–9 days and returned to low Ca medium thereafter. 1 ml of medium was chromatographed on Sephadex G-100 (2.5 × 100 cm column, 0.2 M NH_4OAc, pH 4.7). Values are the counts per minute per gland of each medium for A, B, and C fractions as defined in Fig. 2.



FRACTION	A	B	C	D
BIOASSAY T/C	1.04 ±09	1.56 ±0.13	1.86 ±0.21	1.20 ±0.10

FIGURE 4 Chromatograph of C fraction on Sephadex G-25. 5 mg of C fraction from low calcium cultures of the experiments shown in Table IV were pooled, relyophilized, and chromatographed on Sephadex G-25 (1.5 × 70 cm column, 0.01 M HOAC). The indicated fractions were pooled, lyophilized, and bioassayed at a concentration of 10 μg/ml. Bioassay data are mean ±SE for treated/control ratio for four pairs of bone cultures. Both B and C significantly stimulated resorption ($P < 0.05$) but B contained 3 times as much material by weight.

fraction from rat parathyroid gland culture medium, presumably containing rPTH. Only one showed a precipitin line and this reaction subsequently disappeared. Several sera were found to inhibit the stimulation of bone resorption by both bPTH and rPTH in tissue culture (Table V). Moreover, guinea pig anti-bPTH serum, when injected intravenously in intact rats, caused a rapid decrease in serum calcium concentration which persisted for 10 hr (Fig. 5).

Antibody isolation. On the assumption that the ability of high concentrations of guinea pig anti-bPTH serum to inactivate rPTH was due to binding, a method for antibody isolation of rPTH was tested. Incubation of normal guinea pig serum with a pool of labeled C fraction material at pH 7 did not alter the elution of labeled material on Sephadex G-200 but after incubation with anti-bPTH serum approximately half the radioactivity eluted as high molecular weight material, presumably antigen-antibody complex (Fig. 6). When this fraction was rechromatographed on Sephadex G-50 at pH 7, the radioactivity eluted with the large molecular weight protein, but in 1 N acetic acid the complex was apparently dissociated and the radioactivity migrated as low molecular weight material (Fig. 7). Incubation of the complex with unlabeled bPTH at pH 7 resulted in a displacement of some previously bound radioactivity. When the sharp C fraction peak illustrated in Fig. 3 was incubated with anti-bPTH serum and chromatographed on Sephadex G-50 at pH 7.0, 80% of the label eluted with the serum protein as a high molecular weight complex. When this complex was lyophilized and rechromatographed on Sephadex G-50 at low pH, a biologically active low molecular weight labeled fraction was recovered (Fig. 8, Table III) although there was loss of activity compared with the original C-II fraction.

DISCUSSION

The present studies were undertaken to relate two previous sets of observations: (*a*) that rat parathyroid glands in organ culture could secrete biologically active hormone into the medium (4) and (*b*) that the glands could take up radioactive amino acids from the medium and secrete labeled low molecular weight proteins (5). Both functions were controlled by the calcium concentration in the medium and it seemed likely that the labeled low molecular weight material was rat parathyroid hormone (rPTH) synthesized in culture. The present results confirm this supposition. Not only were the radioactive protein and biologically active hormone found to migrate together on Sephadex G-100 and G-25 columns, but convincing evidence for identity was obtained using an antibody isolation technique. This technique was based on the finding that high concentrations of serum from guinea pigs immunized with bovine PTH (bPTH) could cross-react with rPTH sufficiently to inhibit the action of rPTH on bone in tissue culture and

TABLE V
Inhibition of PTH-Stimulated Bone Resorption by Guinea Pig Anti-bPTH Serum

Experiment	Hormone	Serum	^{45}Ca release T/C ratio
I	bPTH, 0.3 μg/ml	Normal GP, 1/10	1.50 ±0.07
	bPTH, 0.3 μg/ml	GP-E5, 1/10	0.92 ±0.07*
	bPTH, 0.3 μg/ml	GP-E7, 1/20	1.00 ±0.05*
	bPTH, 0.3 μg/ml	GP-E5, 1/100	1.31 ±0.26
	Rat parathyroid culture medium	Normal GP, 1/20	2.49 ±0.02
	0.4 glands/ml	GP-E7, 1/20	1.19 ±0.03*
II	G-25rPTH, 10 μg/ml	Normal GP, 1/20	1.64 ±0.05
	G-25rPTH, 10 μg/ml	GP-A4, 1/20	1.09 ±0.04*
	G-25rPTH, 10 μg/ml	GP-A4, 1/100	1.59 ±0.08

Assays were performed in Eagle's medium with 50% rat serum. Normal or immune guinea pig serum was used as indicated. G-25 rPTH was a pool of fractions B and C from the chromatogram shown in Fig. 4. Values are mean ±SE for treated/control ratio for ^{45}Ca release for four pairs of bone cultures. Underlined values significantly different from 1.0 at $P < 0.01$.
* Significantly different from value with normal GP serum, $P < 0.05$.

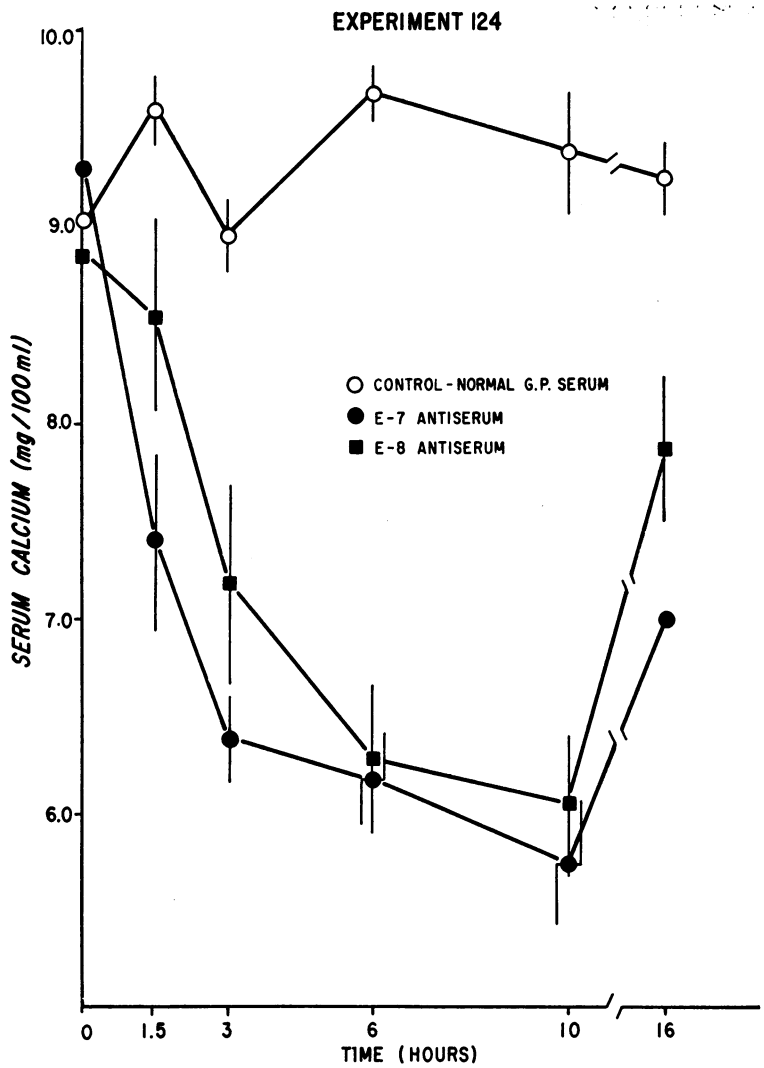


FIGURE 5 Effect of intravenous injection of guinea pig anti-bPTH serum on serum calcium concentration in normal rats. Control rats were injected with 1 ml of a pool of serum from normal guinea pigs. Treated rats received 1 ml of serum from a guinea pig immunized with bPTH. Two samples of serum were tested. Points are the mean and vertical lines the SE of the serum calcium concentration for three to five rats.

to decrease serum calcium concentration in intact rats. However, titer and binding affinity of the antisera for rPTH was not sufficient for a radioimmunoassay.

When biologically active fractions containing labeled low molecular weight protein were incubated with anti-bPTH serum at neutral pH, some of the radioactive material eluted as a high mloecular weight complex. This complex could be dissociated and separated by rechromatography at low pH, and labeled biologically active rPTH isolated. Only a small amount of labeled rPTH was recovered by the antibody isolation technique in the present experiments. The hormone could not be obtained in a pure state because it was necessary to coat the column collection tubes with albumin to prevent loss of active material to the glass. Hence it is not yet possible to determine the biological activity of isolated rPTH on a weight basis. A rough calculation of specific biological activity can be obtained, based on these assumptions. (*a*) the low molecular weight fraction isolated after several days in tissue culture is synthesized from an intracellular labeled amino acid pool which has the same relative specific activity as the radioactive amino acids in the medium; (*b*) rPTH has an amino acid composition similar to bPTH (13), and (*c*) the

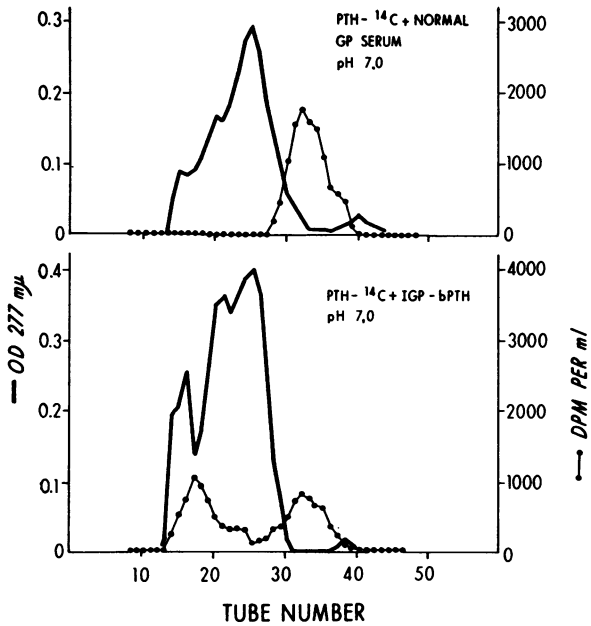


FIGURE 6 Antibody separation of labeled PTH. The active labeled material isolated on Sephadex G-25 (Fig. 4) was used. The C fraction was incubated in 2 ml of buffer containing 0.2 ml of normal or immune (IGP-bPTH) guinea pig serum for 72 hr at 4°C and chromatographed on Sephadex G-200 (1.5 × 40 cm column, 0.2 M NH_4OAc at pH 7. Tubes 15–22 were pooled, lyophilized, and used for further studies (see Fig. 7).

amino acid content of serum from thyroparathyroidectomized rats used in these experiments is the same as that reported previously for normal rats (14). The high specific activity radioactive amino acid mixture added would not appreciably alter this amino acid content. From these assumptions the specific radioactivity of rPTH in the experiment shown in Table III would be 2000 cpm per μg of hormone. Hence, the antibody isolated material (C-II-AI) was assayed at approximately 1.2 μg of rPTH per ml of medium and this had a biological effect similar to that of purified bPTH at a concentration of 1 μg/ml. If the antibody isolated material included biologically inactive rPTH which was immunologically reactive, this would result in an overestimation of the concentration of rPTH. On the other hand, if a large proportion of the amino acids used to synthesize rPTH came from unlabeled precursors, the concentration would have been higher than 1.2 μg/ml.

Regulation of synthesis and secretion of parathyroid hormone by calcium has also been demonstrated for tissue cultures of bovine parathyroid glands by Sherwood, Hermann, and Bassett (6) and Targovnik and Sherwood (15). Recently Hamilton and Cohn (16) found that the calcium concentration in the medium controlled the synthesis of parathyroid hormone by bovine parathyroid slices in vitro, but there was no secretion in this system.

The mechanism of calcium control remains unknown. Changes in calcium concentration in the medium directly

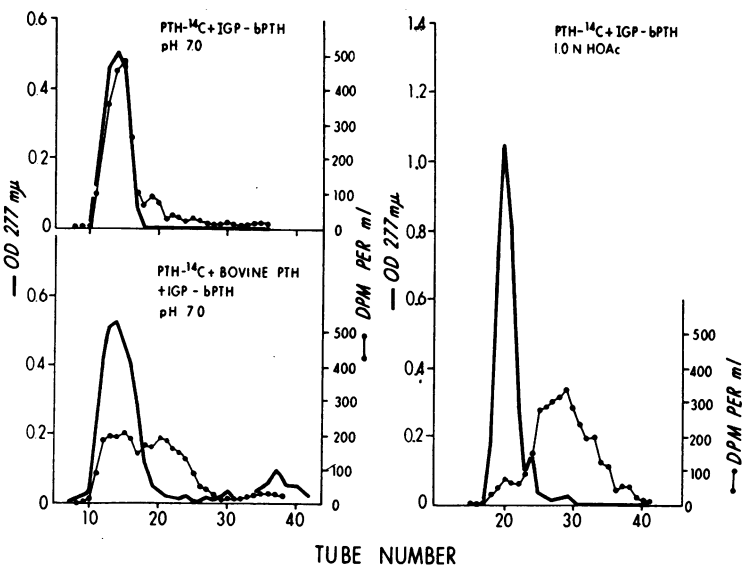


FIGURE 7 Chromatography of rPTH-antibody complex on Sephadex G-50. Fractions 15–22 of the antigen-antibody complex isolated on Sephadex G-200 (Fig. 6) were lyophilized; aliquots were dissolved in 0.2 M NH_4OAc, pH 7, or in 1 N HOAC, pH 2.6, and rechromatographed on Sephadex G-50 columns (1.5 × 44 cm) using the same buffers. One aliquot was incubated with bovine PTH (10 μg/ml) at 4°C before rechromatography at pH 7 as indicated.

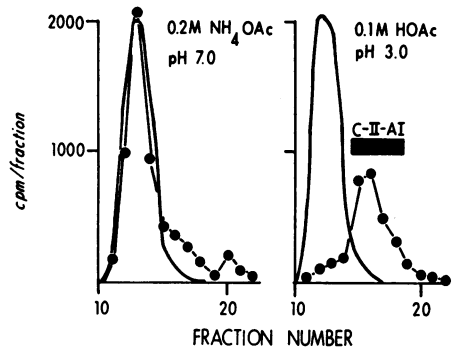


FIGURE 8 Antibody separation of labeled, biologically active rPTH. An aliquot of fraction C-II (Table III) representing the secretion of 10 parathyroid glands was incubated in 5 ml of buffer containing 0.5 ml of A_4 guinea pig serum for 72 hr at 40°C and chromatographed on Sephadex G-50 (1.5 × 86 cm column, 0.2 M NH_4OAc, pH 7). Fractions 11–15 were then lyophilized and rechromatographed on Sephadex G-50 (1.5 × 81 cm column, 0.1 N acetic acid, pH 3) as indicated and fractions 15–18 (C-II-AI, Table III) lyophilized and bioassayed.

affect the uptake of amino acids and this could control hormone synthesis indirectly (7). There may also be important intracellular effects of calcium on protein synthesis, although we were unable to demonstrate acute effects of calcium on protein synthesis after the glands are labeled with a pulse of leucine-^{14}C (17).

In the present experiments only the effects of calcium were examined, but it is clear that magnesium can also effect parathyroid gland metabolism (6, 13, 15). The methods described here might be useful in analyzing the effects of these ions and other factors on parathyroid gland metabolism and for detecting any differences in the control of hormone synthesis and secretion.

ACKNOWLEDGMENTS

We wish to thank Miss Alicia Bukowski, Mr. Steven Gorton, and Miss Ingrid Niemann for their assistance.

This work was supported by U. S. Public Health Service Grant AM 06205. Dr. Au is the recipient of a Pharmaceutical Manufactures Association Foundation Faculty Development Award in Clinical Pharmacology.

REFERENCES

1. Au, W. Y. W., and L. G. Raisz. 1968. Parathyroid hormone (PTH) secretion in organ culture. *Pharmacologist.* **10:** 264.
2. Copp, D. H., and A. G. F. Davidson. 1961. Direct humoral control of parathyroid function in the dog. *Proc. Soc. Exp. Biol. Med.* **107:** 342.
3. Sherwood, L. M., G. P. Mayer, C. F. Ramberg, Jr., D. S. Kronfeld, G. D. Aurbach, and J. T. Potts, Jr. 1968. Regulation of parathyroid hormone secretion: proportional control by calcium, lack of effect of phosphate. *Endocrinology.* **83:** 1043.
4. Raisz, L. G. 1963. Regulation by calcium of parathyroid growth and secretion in vitro. *Nature (London).* **197:** 1115.
5. Raisz, D. G., W. Y. W. Au, and P. H. Stern. 1965. Regulation of parathyroid activity. *In* The Parathyroid Gland: Ultrastructure, Secretion and Function. P. J. Gaillard, R. V. Talmage, and A. M. Budy, editors. University of Chicago Press. 37.
6. Sherwood, L. M., I. Hermann, and C. A. Bassett. 1970. Parathyroid hormone secretion in vitro: regulation by calcium and magnesium ions. *Nature (London).* **225:** 1056.
7. Raisz, L. G. 1967. Effects of calcium on the uptake and incorporation of amino acids in the parathyroid glands. *Biochim. Biophys. Acta.* **148:** 460.
8. Raisz, L. G. 1965. Bone resorption in tissue culture. Factors influencing the response to parathyroid hormone. *J. Clin. Invest.* **44:** 103.
9. Raisz, L. G., and I. Niemann. 1969. Effect of phosphate, calcium and magnesium on bone resorption and hormonal responses in tissue culture. *Endocrinology.* **85:** 446.
10. Aurbach, G. D., and J. T. Potts, Jr. 1964. Partition of parathyroid hormone on Sephadex G-100. *Endocrinology.* **75:** 290.
11. Poland, A. P. 1966. Antisera to parathyroid hormone: biological and immunological studies. Thesis for M.S. in Pharmacology. University of Rochester, Rochester, N. Y.
12. Roth, S. I., and L. G. Raisz. 1966. The course and reversibility of the calcium effect on the ultrastructure of the rat parathyroid gland in organ culture. *Lab. Invest.* **15:** 1187.
13. Potts, J. T., Jr., H. T. Keutmann, H. Niall, L. Deftos, H. B. Brewer, Jr., and G. D. Aurbach. 1968. Covalent structure of bovine parathyroid hormone in relation to biological and immunological activity. *In* Parathyroid Hormone and Thyrocalcitonin (Calcitonin). R. V. Talmage, and L. F. Bélanger, editors. Excerpta Medica Foundation, Amsterdam. 44.
14. Scharff, R., and I. G. Wool. 1964. Concentration of amino-acids in rat muscle and plasma. *Nature (London).* **202:** 603.
15. Targovnick, J. H., and L. M. Sherwood. 1969. Regulation by calcium and magnesium of parathyroid hormone (PTH) production in vitro. 51st Meeting of the Endocrine Society, June 27–29. 102.
16. Hamilton, J. W., and D. V. Cohn. 1969. Studies on the biosynthesis in vitro of parathyroid hormone. I. Synthesis of parathyroid hormone by bovine parathyroid gland slices and its control by calcium. *J. Biol. Chem.* **244:** 5421.
17. Raisz, L. G. 1969. Parathyroid gland metabolism. *Arch. Intern. Med.* **124:** 389.