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Tests on Three Antisera and Subsequent Development of Radioimmunoassay for Different Regions of Human Parathyrin

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Summary

Three antisera (Ab), two raised in sheep and one in a goat, with determinants for different parts of the human parathyrin (hPTH) molecule have been tested. One sheep antibody (Ab S-469 VI) recognised only C-regional fragments of hPTH (C-hPTH), the other (Ab S-478 VI) both C- and N-regional fragments (C+N+hPTH), whilst the goat antibody, raised against 1-34 hPTH (Ciba) was specific for N-regional fragments (N-hPTH). Kinetic studies showed that the differing affinities of Ab S-469 VI and Ab S-478 VI antibodies for bovine PTH (bPTH) and C-hPTH made it possible to develop sensitive assays giving results in as little as 8 hr after receipt of blood, and a routine assay giving results within 30 hr. Although the numerical PTH levels from the assay were different, their ability to distinguish between normals and patients with hyperparathyrinemia (HPT) was similar. Studies on the labelling and separation of ^{125}I-bPTH led to a tracer with a shelf life exceeding 3 months. Correlation between assays using Ab S-469 and Ab S-478 was good, although exceptions occurred, which may have been due to the differing specificity of the antisera.

Comparison of the original 7-day assay with the 24 hr assay using Ab S-469 VI in 109 patients gave a correlation coefficient r = 0.957, and using Ab S-478 in the 24 hr assay in 81 patients a correlation coefficient r = 0.934. The results in the 24 hr assay with Ab S-469 VI were 3 times higher and those from the 24 hr assay with Ab S-478 VI 20% higher than in the 7 day assay. The normal ranges in the 7 day and both 24 hr assays were also different.

The clinical value of the N-hPTH assay has not been fully established but is useful in explaining some anomalous results obtained in the C- and C+N-hPTH assays, in which the non-biologically active PTH fragments were within the normal range for the assay, but in which the N-hPTH level, i.e. the biologically active part of the PTH molecule, was significantly elevated.

Key-Words: *Human Parathyrin Antisera – RIA – C-Regional Fragments – N-Regional Fragments – C- and N-Regional Fragments*

Introduction

The main disadvantage of many published PTH assays is the long incubation time required (*Arnaud, Tsao* and *Littledike* 1971; *Berson* and *Yalow* 1963; *Mallet* and *Brunelle* 1975; *Almquist, Hjern* and *Wästhed* 1975; *Sinha, Queener* and *DeMoor* 1975; *Hehrmann, Wilke, Nordmeyer* and *Hesch* 1976). This study was undertaken to examine the possibility of assay for C-hPTH, C+N-hPTH and N-hPTH which would give results in the minimum time without endangering their clinical value. The introduction of a "same-day-results" assay was envisaged only to help clinicians in emergency cases in which HPT was suspected and such an assay, when combined with an angiographic study of the thyroid region would assist in localizing an adenoma if present, thus helping the surgeon.

As the antisera were raised against undefined preparations of non-human PTH in the case of S-469 VI and S-478 VI, studies were made with human and bovine PTH to characterise the binding kinetics for these assays.

Materials and Methods

1) Antibodies used

Ab S-469 with C-regional acitvity and S-478 VI with C+N-regional activity were raised in sheep against a mixture of bPTH and pPTH and have been described in detail elsewhere (*Hehrmann, Wilke, Nordmeyer* and *Hesch* 1976; *Hehrmann, Nordmeyer, Wilke* and *Hesch* 1977). Both were obtained from Dr. *R. Hehrmann* and Prof. *R.D. Hesch* in Hannover.

Ab-"Pinkey" was raised in a goat against the 1–34 hPTH synthetic peptide from Ciba, Basel, Switzerland, and was obtained from the European Parathyroid Study Group (EPSG) via Prof. *R. Ziegler* in Ulm, Germany.

All Ab were diluted with 0.05 M Barbital Buffer, pH 7.5 containing 1% human serum albumin and 12.5 Antiplasmin Units (APU) of Aprotenin/ml (Trasylol®-Bayer or Antagosan®-Behringwerke) (Buffer 2).

2) Standards and Tracers

bPTH (Inolex, Chicago) was used as standard (Lot No. 1508 B 002) and for labelling (Lot. No. 1515 A 001). A further lot (Lot. No. 1515 B 001) proved to be unsuitable for tracer preparation, due to a high unspecific binding. Standard and tracer for the N-regional assay was prepared from the 1-34 hPTH synthetic peptide from Ciba.

"C-hPTH Standards" consisted of serial serum dilutions from a patient with a secondary HPT calibrated against the S-469 50Q assay described below. All standard curves were made up in PTH-free serum (PTHFS) obtained from a parathyroidectomised patient.

Tracer was prepared by a modification of the method described by *Greenwood* et al. (*Wood* and *Marschner* 1978).

Desalting of the tracer was carried out at room temperature a Sephadex G-10 column and further purification was carried out at 4 °C on Ultragel (LKB) AcA-54 (*Wood, Marschner* and *Scriba* 1978).

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Table 1 Main characteristics of 7 assays described

Characteristic	Assay S-469 50 CL	S-469 50 L	S-469 50Q	S-478 90Q	S-469 50SQ	S-478 90SQ	N-90Q
Antibody initial dilution 1:	20,000	20,000	14,000	2,400	14,000	2,400	25,000
Serum volume µl	50	50	50	90	50	90	90
Incubation time pre- + tracer-incubation hr.	96 + 72	96 + 72	18 + 6	18 + 6	0 + 6	0 + 6	18 + 6
Tracer Bq/tube	50	170	330	330	500	500	330
100.Bo/T	30–35	30–35	15–20	15–20	12–17	12–17	55–65
Normal range ng eq/ml	< 0.28–0.5	< 0.28–0.5	< 0.66–1.5	< 0.24–1.0	+- - - -	+- - - -	< 0.05–0.15
Standard curve range ngbPTH/ml	0.19–12.5	0.19–12.5	0.19–12.5	0.19–12.5	++0.15–10.0	++0.15–10.0	+++0.07–10.0

+ Not determined ++ng eq hPTH/ml +++ng/ml 1–34 hPTH

The fractions from the AcA 54 column with the highest maximum binding (B_0) and lowest unspecific binding (N) were diluted to give about 10 KBq/ml, with Buffer 2. These tracer solutions had a useable shelf-life of more than three months; rather longer than the 9 days reported elsewhere (*Almquist, Hjern* and *Wästhed* 1975).

The elution pattern from the AcA-54 column was stable, the variation of the position of the immunoreactive peak being over a 12 month period 183 ± 3.0 ml, using the same column (n = 18 column-runs).

3) Assay Systems

a) Assay Codes
The following code system is used in the rest of this paper. Each assay consisted of a 3-part code. The first letter and number denoted the antiserum used e.g. S-469 xxx, the second figure the volume of serum used e.g. S-469 50x and the final letter(s) the separation method and length of assay, C for charcoal separation, L. for 7 day assay Q for 24hr-assay and SQ for same-day-assay. The latter 3 assays all used polyethylene glycol for separation of bound free hormone. As an example S-478 90Q – assay with Ab S-478, 90 µl serum separated with polyethylene glycol and having a 24 hr total incubation.

b) Original Assay – S-469 50 CL (Table 1)
This was the original assay in routine use. 50 µl serum and 200 µl Ab were preincubated for 4 days at 4 °C. 1000 µl dextran charcoal (2.5 g Dextran T-70 (Pharmacia) + 25 g Norit-A (Sigma) in 1000 ml Buffer 2) was added, and after centrifugation (2500 g, 15 min) the supernatant transferred to a clean tube and counted for 5 min in a gamma-counter. The results in this and all subsequent assays were then worked out using a spline function (*Marschner, Erhardt* and *Scriba* 1975; *Marschner, Dobry, Erhardt, Landersdorfer, Popp, Ringel* and *Scriba* 1974) and off-line techniques by computer (Siemens 404/3). The range of the standard curve was 0.19 –12.5 ng bPTH/ml, B_0 was measured 10 times and the standard curve points and sera 5 times.

c) Modified Long Assay – S-469 50L (Table 1)
The assay was identical with S-469 50CL but more radioactive tracer was added to each tube and polyethylene glycol (PEG) (12% solution in 0.18 M NaCl) was used to separate the bound and free hormone. The resulting precipitate was counted for two min.

d) Quick Assay – S-469 50Q, S-478 50Q and S-478 90Q (Table 1)
These assays all had a preincubation of 18 hr and after addition of tracer, a second 6 hr incubation before separation with PEG and counting.

e) Same-day Assays – S-469 50SQ and S-478 90SQ (Table 1)
These assays only had one incubation step where Ab, tracer and serum were incubated together at 4 °C for 6 hr. Only a C-hPTH standard curve could be used due to the binding kinetics of the Ab with bPTH and C-hPTH (see below for results of the kinetic studies). Other details were the same as for the Quick assays.

f) N-regional Assay – N-90Q (Table 1)
The details for this assay are identical with those for the S-478 90Q assay except that the standard curve used the 1-34 hPTH Ciba peptide.
In all assays using PEG separation, 100 µl carrier serum was added to each tube directly before the PEG in order to smooth-out the unspecific binding in each serum.

4) Kinetic Studies
Kinetic studies were carried out with S-469 VI and S-478 VI to investigate the different affinity to bPTH and C-hPTH with time. B_0, bovine standards of 1 and 3 ng/ml and human sera with 1 and 3 ng/ml (measured by the S-469 50L assay) were used in this study. Bovine standards were made up in PTHFS. The kinetics were studied with and without a preincubation of 18 hr before tracer addition and were carried out over a 7 or 10 day period.

5) Establishment of Normal Range and Correlation Studies
Normal ranges were obtained for the following 4 assays: S-469 50L, S-469 50Q, S-478 90Q and N-90. Correlations were made between the first three, and their ability to differentiate between normal collective and patients with HPT tested. The stability of binding of tracer at each point on the standard curve was tested for each of the above assay as well as for S-469 50L. In these studies no correction was made for the age of the tracer which was from 1 to 110 days old.

Results

1) Tracer Preparation

a) ^{125}I-bPTH

Figure 1a shows the elution-profils of the iodination mixture desalted on a Sephadex G-10 column. Here the protein:iodide count-ratio was usually between 3:1 and 4:1.

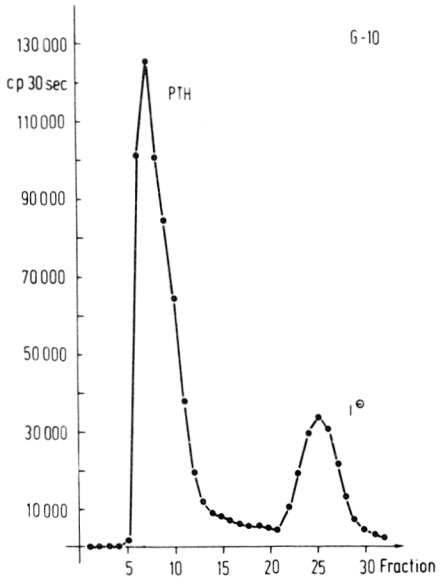

Fig. 1a Elution profile of Sephadex G-10 column using buffer 2 as eluant. Fractions contained 500 µl. The protein peak eluted with the void volume

Only the first of the two closely separated peaks has any immunoreactivity. The B_0 of the material from the first major peak (Peak 3) was 52% in this example and in the second peak (Peak 4) only 5%. The unspecific binding was under 3% in both cases. Peak 4 showed no immunoreactivity with the "Pinkey" Ab thus ruling out an N-regional peptide. The tracer so produced, had a shelf-life exceeding 3 months. The

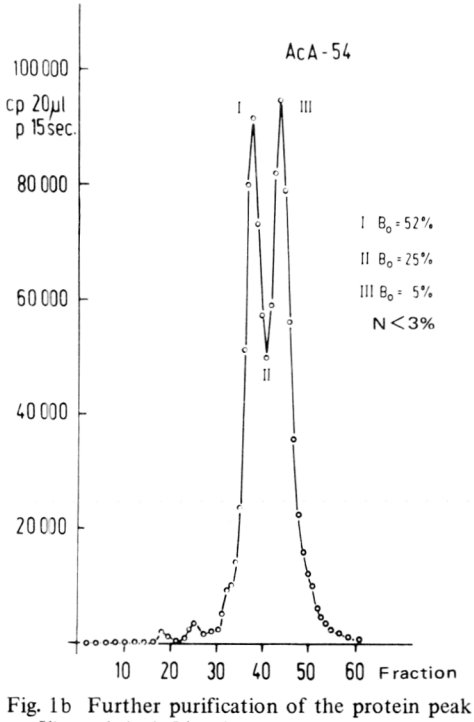

Fig. 1b Further purification of the protein peak from 1a on Ultrogel AcA-54 column

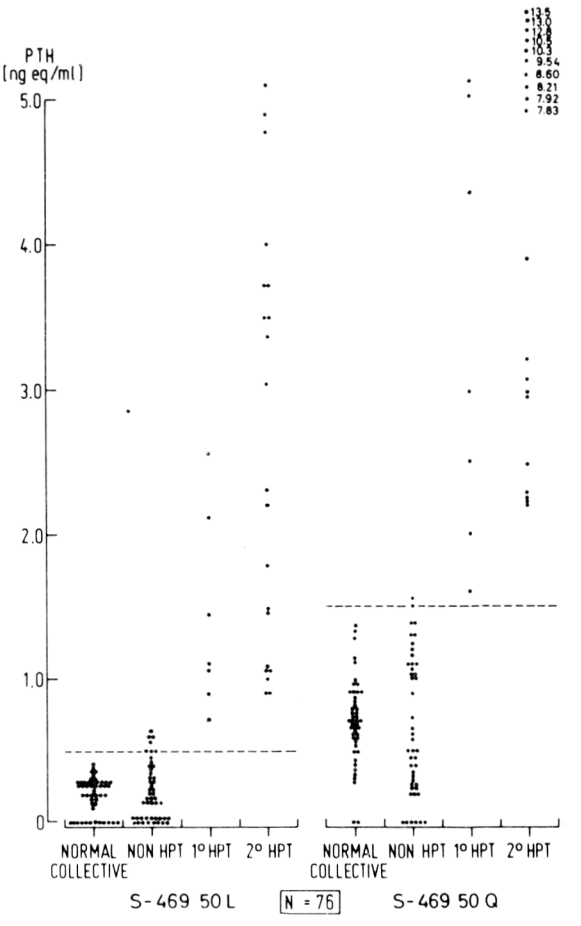

Fig. 2a Correlation between S-469 50L and S-469 50Q assays in 109 patients

decrease in B_0 was under 3% as was the increase in N. The quantity and quality of the tracer appears to be greater than reported elsewhere (*Hehrmann, Wilke, Nordmeyer* and *Hesch* 1976).

b) ^{125}I-1-34 hPTH

The elution profile of the iodinated 1-34-hPTH on the Sephadex G-10 desalting column was similar to Fig. 1a. The iodide:protein count-ratio was normally between 1:1 and 1.5 : 1. The rechromatography on Ultrogel AcA-54 gave a damage-peak at the void volume and a single immunoreactive peak appearing at approximately the same place as human calcitonin and β-endorphine indicating an iodinated monomeric form of the peptide.

2) Assays

a) Characteristics

The results given by the S-469 50CL and S-469 50L assays were the same, but the addition of higher tracer amounts abolished the "hook-effect" (*Joel, Schönberg, Ilg* and *Keller* 1974) often seen in the S-469 50-CL assay. Addition of tracer in excess of 350 B_q/tube

Table 2 Results of an angiographic study measured in 7 assay systems of a patient with 2.HPT. Control sera show the variation in the absolute values in the different assay systems

Control Sera	Assay S-469 50 L	S-469 50Q	S-469 50SQ	S-478 50Q	S-478 90Q	S-478 90SQ	N-90Q
	ng eq/ml						ng/ml
RKS 1771	0.41	1.11	0.12	0.27	0.21	0.17	- - -
RKS 1772	0.79	1.94	0.40	0.66	0.55	0.53	- - -
RKS 1773	3.04	7.83	4.25	4.33	3.35	4.80	- - -
Angiography Patient Bo							
r. int. jug. vein upper	0.90	2.12	0.45	1.54	1.27	1.28	0.06
l. int. jug. vein upper	0.95	2.20	1.00	1.67	1.38	1.21	0.05
r. int. jug. vein lower	0.75	2.22	0.82	1.86	1.42	1.46	0.09
l. int. jug. vein lower	1.05	2.28	0.95	1.58	1.26	1.39	0.11
thyroid ima vein	4.89	7.97	3.02	6.92	5.98	5.24	0.29
int. thyroid vein	4.05	7.90	2.13	6.00	5.95	4.32	0.28
l. subclavian vein	1.16	2.11	0.91	1.67	1.42	161	0.12

(freshly labelled tracer) caused little increase in B_0 but significantly increased the unspecific binding, thus flattening the standard curve, seen in the increasing value of the 50% intercept. The range covered by the standard curve was the same for both S-469 50CL and S-469 50L assay. PEG separation increased precision thus making it possible to set up the sera in triplicate instead of in pentuplicate as in S-469 50CL. In all patients who had disproteinemia an unspecific binding was necessary to correct the bound counts. In effect, this was seen in about 10% of all patients.

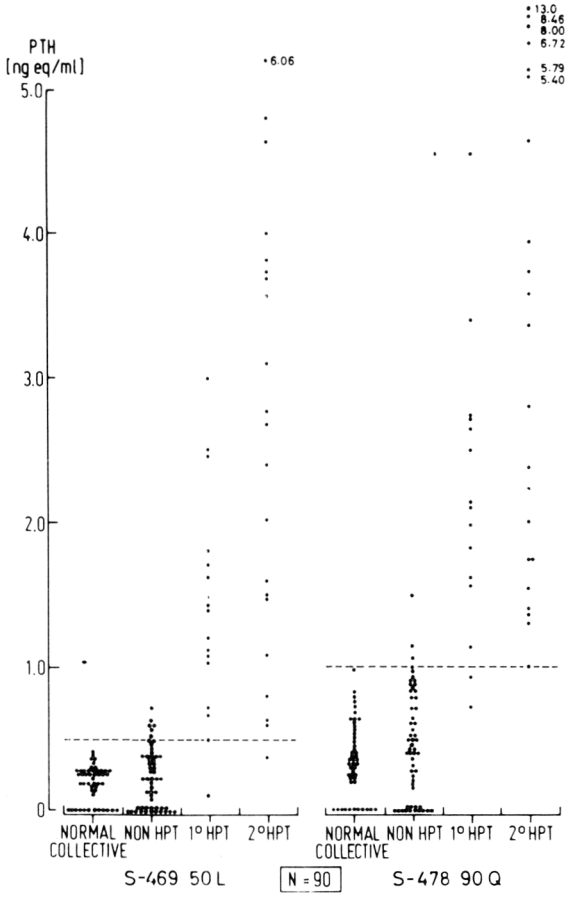

Fig. 2b Correlation between S-478 90Q and S-478 50L assay in 81 patients

The values from the S-469 50Q assay were 3 times higher than those from the S-469 50L assay. This effect was seen throughout the concentration range. Fig. 2a shows the correlation between these two assays on 109 patients. The S-478 50Q assay was soon abandoned for the S-478 90Q assay as the latter gave better precision. The values from the S-478 90Q assay were around 20% higher than those from the S-469 50L assay (Fig. 2b). The correlation between these assays was highly significant. The results used in the correlation studies were taken from parallel assays set up at the same time, 3 or 4 such assays being used in the construction of Figures 2a and 2b.

The same-day assay S-469 50SQ and S-478 90SQ were developed for emergency use. The characteristics of the 4 main assays are shown in Table 1.

b) Diagnostic Use

Table 2 shows the results in 7 assays of an angiographic study of a patient with suspected secondary HPT, together with values from precision control sera. The clinical value of the results is the same in all cases. Although the numerical values are often very different.

The N-regional assay was performed routinely on over 300 samples which came into the laboratory for "hPTH" assay to assess its clinical usefulness. Certain patients were found who had normal or only slightly elevated parathyrin levels when assayed in the S-469 50L or S-478 90Q assays, but who had definite clinical signs of HPT. Many of these patients showed an elevated N-90Q assay value between 3 and

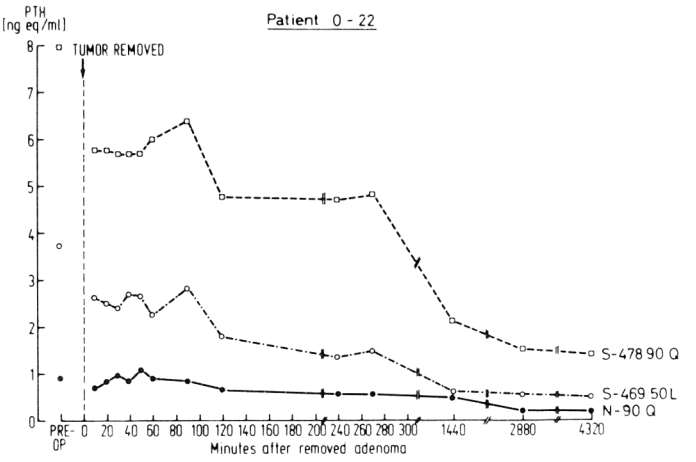

Fig. 3 Patient 0–22 showing immunoreactive PTH serum levels before and up to 3 days after removal of an adenoma, as measured in 3 assay systems

10 times the normal values. One patient was found to have an adenoma which secreted N-regional immunoreactive PTH and Figure 3 shows the levels of PTH measured in three assays before and up to 3 days after removal of the adenoma.

To assess the usefulness of the assays in their ability to differentiate between a normal population and patients with HPT, parallel studies were made between the S-469 50L and S-469 50Q assays in 76 patients (Fig. 4a) and between S-469 50L and S-478 90Q assays in a further 90 patients (Fig. 4b). The results showed that the assays have the ability to differentiate between normals and HPT and also between patients with calcium metabolism disorders due to other causes and HPT.

3) Standard Curve Characteristics and Reproducibility

Table 3 shows the standard curve binding characteristics of the S-469 50L, S-469 50CL, S-469 50Q and S-478 90Q assays, together with sensitivity and 50% intercept values for each assay. Table 4 shows the same for the N-90Q assay. The reproducibility of binding is seen, and no correction has been made for the age of tracer. The change from charcoal to PEG for separation of bound and free hormone has minimal effect on the binding but has a lower standard deviation.

The S-469 50Q assay was less sensitive and had a higher 50% intercept but when seen in context with the 3 times higher results, the assay was comparable to the S-469 50 L assay. The binding characteristics

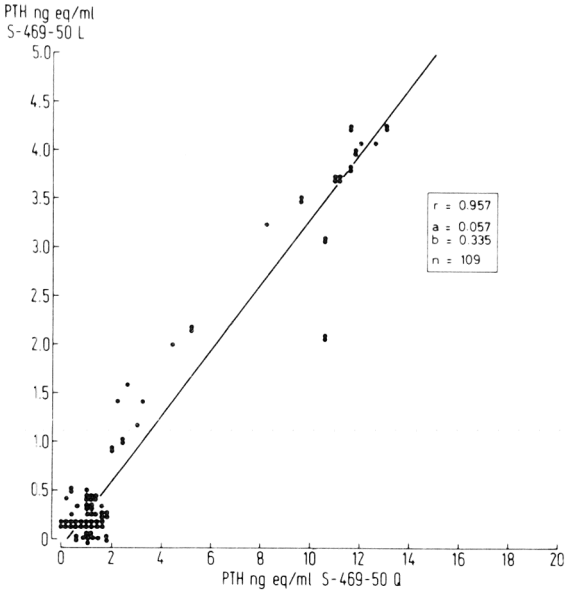

Fig. 4a Comparison of results between S-469 50L and S-469 50Q assays in 47 normal persons and 76 patients with calcium metabolism disturbances

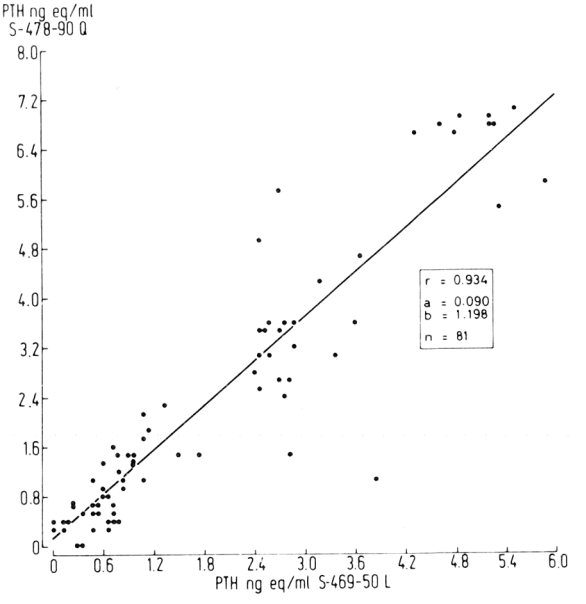

Fig. 4b Comparison of results between S-478 90Q and S-469 50L assays in 48 normal persons and 90 patients with calcium metabolism disorders

Table 3 Mean and standard deviation (s.d.) of the percent binding of the standard curve points, together with the sensitivity, 50% intercept and number of assays from which the data was obtained

Standard ng bPTH/ml	S-469 50CL %Bo x s.d. CV%	S-469 50L %Bo x s.d. CV%	S-469 50L %Bo x s.d. CV%	S-469 50Q %Bo x s.d. CV%	S-478 90Q %Bo x s.d. CV%
0.19	96.3 ± 1.74 1.82	97.2 ± 1.84 1.89		98.0 ± 0.62 0.64	93.7 ± 2.89 3.09
0.39	90.9 ± 1.78 1.95	92.4 ± 2.06 2.23		96.1 ± 1.23 1.28	88.5 ± 3.29 3.72
0.78	82.9 ± 3.94 4.70	84.6 ± 3.67 3.67		92.8 ± 2.00 2.16	78.2 ± 4.33 5.55
1.56	67.5 ± 6.19 9.16	68.1 ± 2.76 4.06		83.2 ± 2.28 2.75	63.0 ± 5.48 8.69
3.12	39.1 ± 6.67 17.1	40.8 ± 4.28 10.5		65.0 ± 4.62 7.11	44.6 ± 4.68 10.4
6.25	15.6 ± 2.87 18.4	18.2 ± 2.41 18.2		36.9 ± 1.08 2.93	26.9 ± 4.97 18.4
12.5	9.0 ± 1.17 13.0	11.4 ± 1.66 14.6		16.1 ± 2.28 14.2	16.5 ± 4.10 24.8
Sensitivity ng bPTH/ml	0.28±0.13	0.28±0.08		0.66±0.16	0.24±0.10
50% intercept ng bPTH/ml	2.39±0.40	2.49±0.21		0.40±0.50	2.60±0.45
No. of Assays	10	10		5	12

Table 4 This shows the same data as Table 3, but for the N-90Q assay

Standard ng 1-34hPTH/ml	Bo% x s.d.	CV%
0.15	81.1 ± 2.13	2.56
0.31	65.8 ± 3.10	4.71
0.62	47.6 ± 3.29	6.92
1.25	30.3 ± 3.20	10.6
2.50	18.0 ± 4.30	23.8
5.00	12.2 ± 3.82	31.2
10.0	8.3 ± 3.50	42.0

Values from 8 assays

of the S-478 90Q assay were slightly different although sensitivity and 50% intercept were similar to S-469 50CL and S-469 50L assays. The curve was not so steep in the S-478 90Q assay but fell away from zero quicker. The N-90Q assay was more sensitive and was able to measure within the normal range, the sensitivity and 50% intercept being about 20% of those for the S-478 90Q assay. As the normal range of this assay was also about 20% of that of S-478 90Q, the two were comparable in this respect.

In all cases, sensitivity was defined as the point of the standard curve lying 3 standard deviations away from the zero standard. All values are the mean and standard deviation of several assays as shown.

4) Kinetic Studies

The kinetic study results are shown in Figures 5a and 5b and highlight the different binding characteristics of S-469 VI for bPTH and C-hPTH. Figure 5a shows the rate of binding of tracer after a 18 hr preincubation expressed as cpm bound.

Table 5b shows the rates of binding when the preincubation is omitted. The results show quite clearly why the S-469 50Q assay delivers higher results than

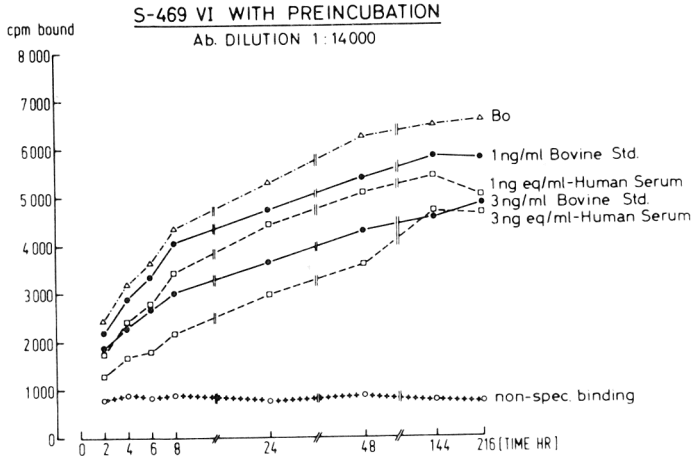

Fig. 5a Ab S-469 kinetic studies showing the difference in the reaction rates of bPTH and hPTH with time, accounting for the difference in results obtained in the long and short assays. Assay with 18 hr pre-incubation

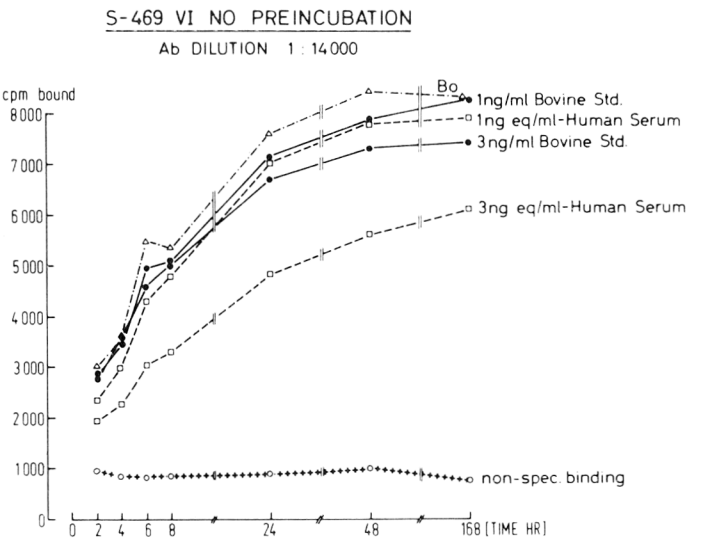

Fig. 5b As Fig. 5a but without the 18 hr pre-incubation. This shows why the same day assay only functions with an hPTH standard curve

the S-469 50L assay, and also why the same-day assay only function with a C-hPTH standard curve. The kinetics for S-478 VI were similar and have been reported elsewhere (*Wood, Marschner* and *Scriba* 1978).

Discussion

This study has demonstrated that a modification of the labelling technique combined with drastic shortening of the assay time has led to assays with the same sensitivity and reproducibility as the original long assays. In the tracer preparation, no trace of „damage" peaks was seen in the elution profiles as reported elsewhere (*Hehrmann, Wilke, Nordmeyer* and *Hesch* 1976) which lead to the question as to whether these are artefacts introduced by the technique used, rather than oxidation products produced by the chloramine-T. The Ultrogel AcA 54 column only functioned at 4 °C.

This labelling technique in producing "long-life" tracer is not only restricted to bPTH but is used regularly in this laboratory in the production and storage of human calcitonin (shelf-life longer than six months) transferrin and β-endorphine.

The introduction of an analogue of the *Wide* technique (*Wide, Nillius, Gemzell* and *Roos* 1973) led to the shortening of the assay without any loss of sensitivity or clinical value of the results. Ab S-469 VI was "serum sensitive" and any attempt to increase the serum amount in the assay led to reduction of B_0 and to a less sensitive assay. This together with the difference in reaction rates between bPTH and C-hPTH with the antibodies raised in sheep have no doubt helped in the development of such short assays, especially the same-day assays. The circulating forms of hPTH are themselves undefined, and are composed of peptides of varying compositions and biological activity (*Berson* and *Yalow* 1968; *Habener, Powell, Murray, Mayer* and *Potts* jr. 1971; *Segre, Habener, Powell, Tregear* and *Potts* jr. 1972; *Canterbury* and *Reiss* 1972; *Canterbury, Levey* and *Reiss* 1973; *Arnaud, Goldsmith, Sizemore, Oldham, Bischott, Larsen* and *Gilkinson* 1973; *Arnaud* 1974; *Silverman* and *Yalow* 1973). In certain pathological conditions the intact 1-84 hPTH is present in serum in relatively small amounts when compared with the degradation products (*Segre, Niall, Habener* and *Potts* jr. 1974; *Purnell, Scholz, Smith, Sizemore, Black, Goldsmith* and *Arnaud* 1974; *Segre, D'Amour* and *Potts* jr. 1976). Bearing these facts in mind, the value of a 1-84 hPTH assay can only be supplementary to the fragment assays at present in use. More important is the introduction of internationally defined preparations extracted from human serum or parathyroid adenomas containing C- and N-regional peptides to act as reference standards in addition to those already axisting for 1–84 hPTH such as the MRC 75/549.

Ab S-469 VI does not react with 1-34 hPTH and Ab S-478 VI only to give a B_0 of 5–10% in the S-478 90Q assay using ^{125}I-labelled 1–34 hPTH as tracer. The ^{125}I-bPTH from Inolex does not react with the "Pinkey" Ab even at an i.d. of 1:10,000 showing that this material is unlikely to be 1–84 bPTH, but more likely a large C-regional peptide or group of peptides. Results from other laboratories (*Hehrmann, Wilke, Nordmeyer* and *Hesch* 1976) have shown that S-469 VI does not react with the 13-34, 18-34 and 23-34 peptides of hPTH, or with the 1-34 hPTH from Beckman. Another observation was that the same-day assay coded S-478 90SQ did not give useable results if the incubation was carried out at room temperature instead of at 4 °C.

The reason for the higher values obtained in the shorter assays for the C- and C+N-regional assays may be due to the fact that the antibodies with the highest avidity for C-hPTH bind first, and the less avid ones bind the bPTH somewhat more slowly eventually giving rise to more counts bound, hence to lower results. In contrast, the values in a 4 hr version of the N-90Q assay were identical with the 24 hr assay, possibly due to the fact the antibody was raised against a human peptide of unique structure, rather than a undefined mixture of bPTH and pPTH of unknown structure and purity. The kinetic studies have shown the reason for the different numerical values for PTH with different incubation times. In patients at risk the short assay were combined with an angiographic study in order to locate the areas of high PTH production. It has been clearly demonstrated in this laboratory that the C- and C+N-regional assays may be just as good as an N-regional one in most cases for these purposes, as shown in Table 2 and the other angiographic studies carried out. The C-assays may be better because of the much larger concentration differences between "background" and "hot" areas.

The use of parathyrin-free serum (PTHFS) for standard curves is imperative in all non-extractive procedures in order to standardise conditions in the standards and serum samples under test. The use of synthetic protein solutions such as albumin or a "synthetic serum" made up of 4% HSA, 1% β-globulins and 2% human γ-globulins was not acceptable as the B_0 and shape of the standard curve were different from that obtained using PTHFS. Equine serum and serum from HPX dogs were also unsuitable for the same reason. The disadvantage of PEG is in its serum globulin concentration dependance but the small number of patients with disproteinemia severe enough to cause false results was so low as not to cause undue hinderance with excessive numbers of unspecific binding tubes – about 10% of all samples received. Unspecific binding tubes should be run with all sera from dialysis patients, as this group has shown the greatest deviation in unspecific binding, which could significantly affect results if not taken into account. All sera with total protein outside the range 60–80 g/liter should be run with an unspecific binding.

The N-regional assay showed values within and above the normal range in several patients with clinically confirmed HPT, as has been reported elsewhere (*Minne, Raptis* and *Ziegler* 1976), and was therefore only used in cases where the other assays had given unclear results.

Tables 3 and 4 have shown that the assays are sensitive and reproducible enough for routine use. The S-478 90Q assay was adapted for routine use because of its wider specificity and also as a result of a meeting of PTH-laboratories in Hannover, Germany, in May 1976 (Chairman Prof. *R.D. Hesch*). This assay has detected all 2. HPT and between 85–90%. 1.HPT patients in the 12 months that this assay has been in routine use and in which time over 1800 patients have been examined.

To conclude, although the assays here described are heterologous, the results which they give allow a rapid and reproducible assay for hPTH to be performed with the accuracy required in the confirmation or rejection of hyperparathyrinaemia.

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